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TUMORS, CYSTS, PITH-BUNDLES, AND FLORAL PROLIFERATIONS IN HELIANTHUS

BY

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TUMORS, CYSTS, PITH-BUNDLES AND FLORAL PROLIFERATIONS IN HELIANTHUS

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In earlier papers in 1911 (*1*, *2*), 1912 (*3*), 1916 (*4*, *5*), 1917 (*6*, *7*), 1920 (*8*), 1922 (*9*, *10*), I stated that I had obtained striking modifications in the structure of various plants by crown-gall inoculations, e. g., abnormal and very rapid cell division even of rather well-developed parenchyma cells, resulting in tumors with marked disorientation of cells and vessels; tumor strands in protoxylem and in cortex; the development of pseudostems in leaves; enormous local thickenings of the wood and cortex in proximity to the tumors; abnormally wide medullary rays; shoots from midribs of tobacco; roots, shoots, and floral organs variously out of place; a stele in the cortex of tobacco; prolepsis of shoots and fasciations.[1] I also showed it to be possible to obtain small tumors on various plants, and steles in the pith of Ricinus, by means of chemical stimuli and in a stimulated begonia numerous trichomes producing shoots. All these writings were accompanied by photographs and photomicrographs illustrating the changes.

Since I began to be interested in these problems, similar results have been obtained in animals by various experimenters, so that we are warranted in concluding that they apply to all living things, and show that a changed environment may tremendously modify heredity.

The following paper is a continuation of these studies.

In 1923 in several heads of *Helianthus annuus* I obtained ray flowers standing in the middle of the head among tubular disk flowers. These heads had been inoculated with the hop strain of the crown-gall organism (*Bact. tumefaciens* S.andT.).

Twice in 1924, on stems inoculated below the flower head but in the upper part of the growing stem using the same organism, I obtained a well-developed vascular cylinder (stele) occupying the center of the pith. In one case this was prolonged upward more than 2.5 centimeters beyond the highest external tumor. One at least of these steles arose from the protoxylem region of the stem. The origin of the other one was not determined.

These findings led to numerous experiments to determine the cause of these abnormalities.

In 1925 more than 100 sunflowers were inoculated in the young flower head on June 18 (rather too late), by needle pricks, using agar streak cultures of the hop strain of *Bact. tumefaciens*. Of these plants 30 per cent produced ray flowers and big green bracts among the tubular flowers, as shown on the plates. Many which did not proliferate the central ray flowers developed crown galls in the head.

On dissection these ray flowers out of place (all of which so far as examined were sterile) were found to be part of stalked or sessile, perfect or imperfect small supernumerary flower heads developed from the middle of the receptacle. Some of these supernumerary small heads were very perfect as on No. 4B, C, No. 6DD, No. 19C and No. 22B. Others had ray flowers and green bracts only on one side, the other side being normal, i. e., not separated from the rest of the tubular flowers.

In addition to the floral anomalies referred to, cysts developed in the stems of a large number of the inoculated plants. Up to this time I had never seen cysts in connection with crown-gall inoculations. By cysts I mean cavities in the pith, lined by a well-defined small-celled membrane. These cysts in the pith were often 1 to 2 feet long, sometimes longer, and were always subtended by groups of vascular bundles (xylem-phloem bundles). Not infrequently these pith bundles were compacted into a well-defined woody cylinder (stele) occupying the center of the stem, often for long distances.

[1] Recently Miss Nellie A. Brown, of my laboratory, has described a very striking and injurious prolepsis of shoots and fasciation on the sweet pea, due to the crown-gall organism. These were received from hothouses in New Jersey and in Ohio. From them *Bact. tumefaciens* was isolated in pure culture and with it the disease was reproduced on the sweet pea in our hothouses (*Phytopathology*, 1927). The disease causes the plants to make a much-branched complex of dwarfed, pale, bunched stems at or near the surface of the earth.

The central steles referred to as obtained in 1924 (Pl. I) contained no suggestion of a cyst, but their center was occupied by more or less disoriented parenchyma cells. On the contrary, in 1925 the stem-pith steles obtained by inoculating the young flower head with the crown-gall organism were hollow, and the cavity was bordered by a regular tissue of cells much smaller than the pith cells. The walls of these cavities were either smooth or granular or sparsely hairy or covered with great numbers of glandular and acicular trichomes similar to those occurring on the torus; that is, on the surface of the plant. These cysts, always subtended by reversed xylem-phloem bundles, must be assumed to be invaginations (implantations) of the meristematic torus, unless it can be shown that xylem-phloem bundles are normal to the pith of the sunflower stem and were the parts stimulated, something I have not been able to discover, and even then it would be hard to account for the lining membrane with its surface hairs and for the reversed xylem-phloem bundles.

Continuous crown-gall tissues were sometimes found in these cysts, but more often interrupted tumors of various sizes. The larger tumors filled and expanded the cysts so as to occupy the whole or the most of the pith, and some of them pushed out the normal xylem-phloem-cortex, rupturing to the surface. Other tumors, and generally the larger ones (Pl. XIII), were of cambial origin. These abnormal xylem-phloem bundles continued down the pith of the stem as far as the tumors extended but not beyond, or not much beyond—that is to say, a distance, as already stated, of 1 to 2 feet or more from the head; or, to express it in another way, corresponding to the development of the plant, as far up as the growth extended following the inoculation.

In all cases, so far as sections were cut and studied, the orientation of the xylem-phloem bundles in the pith steles is the reverse of that in the normal vascular bundles, i. e., their phloem faces the cyst cavity. In many instances the phloem is accompanied by well-developed bundles of hard bast, which are nearer the cyst wall than the phloem bundles (Pl. XXVIII). Occasionally when the bundles are discrete, the xylem entirely surrounds the phloem or the reverse. Beyond the xylem of these long steles, in every direction, is undisturbed pith. In other words, we have here within the stem a second stem or a series of such stems (three to six in some instances) with reversed polarity, as if a portion of the apex of the shoot had turned and grown downward through the middle of itself. Whether it ever really grows downward, and if so to what extent, is a matter yet to be determined. Clearly, most of the extension is upward.

Several problems suggested by these curious results remained to be solved.

First, I wished to know whether the floral proliferations and cysts could be obtained in the absence of tumor irritation, i. e., by simple wounding. I believed they could be. Beginning, therefore, in 1924 a series of young sunflower heads (about 50) were wounded by removing the central part of the torus by means of a small cork borer, but none of the heads produced any central ray flowers.

In 1925 many still younger plants were wounded by means of a scalpel, a small cone-shaped piece of the very young flower head being removed (center of torus and a little pith under it). None of these heads proliferated ray flowers.

The two attempts on a large scale failed, as indicated, but these were not made in exactly the same way as the inoculations and therefore another attempt was made.

After the first crop of sunflowers was out of the way, in 1925, a second planting was made, and 108 of these plants were needle pricked in the young heads on September 3, exactly as for a crown-gall inoculation, but nothing was introduced into the wound except that which might happen to be carried in from the clean surface, certainly no crown-gall organisms. Some of the flower heads were an inch or a little more in diameter when pricked, but the larger number were smaller than this and were in such a very meristematic condition that it was believed some might respond with floral proliferations, cysts, and pith bundles, which proved to be the case.

A second question related to the possible normal occurrence of xylem-phloem bundles in the stem pith of *Helianthus annuus*, since while they appear to be rare in the *Tubuliflorae* to which Helianthus belongs, they occur in many genera of the *Liguliflorae* (Chicoriaceae) generally considered to be the highest type of *Compositae*. I have found no mention of such stem bundles in

literature on Helianthus, and I never observed any in previous years in several hundred sunflower stems cut for other purposes; but, fearing some might have been overlooked, more than 100 control plants were held in 1925, and the stems cut and examined critically, in many places, for pith bundles, but without finding any. They do, however, occur sparingly on the periphery in the expanded pith of the head, but they are without relation, I believe, to the results here described, since their orientation is the same as that of the outer bundles.

Of the 108 plants needle punctured in the young flower heads on September 3 and examined in October, seven developed ray flowers among the tubular flowers. (Pl. XXIX.) In every instance these were the last heads to open either because they were the youngest when pricked or because they developed more slowly than the others. In general in this lot of autumn-grown plants, the flowers opened very soon after they were punctured as if sensing the approach of winter; and the heads and stems never reached anything like the size of those of the first crop (the crown-gall inoculated plants, which were very robust). Undoubtedly punctures made a week or 10 days earlier would have given a higher percentage of positive results. Dissection of the heads and stems of these 108 plants for the presence of cysts and of pith-bundles showed the same results as in the crown-gall inoculated plants. Cysts were found in the stem pith of 40 of these plants. The walls of these cysts were covered either with fine granulations or were lined with many soft white hairs. (Pls. XXXI, XXXII.) These pith cysts were subtended by reversed vascular bundles, separate or united into a stele, exactly as in the case of the crown-gall inoculated plants. Occasionally in the upper part of the stems, as growth continued, the cysts in both groups ruptured to the surface, as long deep grooves, the walls of which became green. The floral proliferations were much less frequent than in the crown-gall inoculated plants, already referred to, but this was probably due, in part at least, to less vigorous growth. In the greater number of the plants the only result from the needle punctures was a solid or hollow mass of scar tissue not subtended by vascular bundles, often dead in the center, and when hollow not lined by any membrane; but sometimes there was an irregular large-cell proliferation into these cavities, such as one often sees in gum pockets. Such cavities are not to be confounded with the cysts, since they had a different origin and are morphologically distinct.

These sunflowers have been grown in our houses and on our grounds for the use of this laboratory since 1915 and nothing of this sort was ever seen before in any of them. It is a coarse-growing, vigorous plant, of Russian origin, I think, usually giving under roomy good conditions a stem 10 to 12 feet high, 2 inches or more in diameter, with very broad leaves and a single head 12 to 14 inches in diameter, with one row of peripheral (sterile) ray flowers and several to many hundred fertile disk flowers. The seeds are dark gray with lighter longitudinal lines, but the strain is not perfectly pure, since in some plants the seeds are of a darker color, and in 1925 in about 10 per cent of the plants all the upper leaf axils gave rise to flower stalks with small disks (2 to 3 inches in diameter), making very showy plants.

DISCUSSION

Vigor of growth evidently had much to do with the success of the experiments, since I got more floral proliferations and cysts from the plants pricked with a sterile needle than I did from another set of 89 crown-gall inoculated plants done at the same time (September 3, 1925). Several factors militated against the success of the latter experiment, the chief of which seems to have been the fact that they were on the north side of a house and made only about one-half as much growth as those in the open ground with full sunlight where the other experiments were conducted.

The cysts may be regarded as the result of implantations of tiny fragments of torus into the tissues under the torus. Here they are anchored (healed on) and elongate with the growth of the stem. This is the simplest explanation. Whether it is the full explanation is another matter. In a case like No. 10B (Pl. XXV) it is hard to believe that the rather remote subtending vascular bundles are a product of the originally implanted tissue, and yet they may be. On the other hand, may they not be the product of very young pith cells abnormally stimulated?

Their regularity would suggest this. If the ray flowers out of place were fertile we might infer that they got an excess of nutrient substances, but as they are sterile, like the normal rays, we may suppose that they are tubular flowers converted into sterile ray flowers through some defect in nutrition. All of the disk flowers of the supernumerary capitula developed a little later than the surrounding tubular flowers.

The results of these experiments confirm and extend earlier ones on other plants—daisy, pelargonium, sugar beet, tobacco, ricinus, tropaeolum, etc. They show that what young cells will become depends to a very considerable extent on how they are treated. A changed stimulus, if not too strong and if applied early enough, leads inevitably to a changed structure. From the experiments here described I have obtained cysts with and without tumors on their walls (without when only needle pricks were used); have shown that cysts and tumors have no necessary connection (something of interest to surgeons and oncologists); have obtained numerous xylem-phloem bundles and steles (with reversed polarity) in the central pith of the stem, otherwise free from bundles; and have produced among the tubular flowers in the center of the sunflower disk, tumors, roots, linear green bracts, yellow ray flowers, broad green bracts like those on the margin of the head, and secondary, sessile or stalked, imperfect and perfect flower heads (supernumerary capitula). Masters, in his Teratology, has figured (and the writer has seen) stalked roses growing out of the center of other roses, quite like No. 4B (Pl. XVI), but nothing is known as to their cause.

SUMMARY

The general conclusion I would draw from these experiments is that many of the common teratological forms in plants and animals are due to parasitic or mechanical traumatic displacements occurring in early life.

Addendum

EXPERIMENTS OF 1926

Delay in the publication of this paper has enabled me to add to it the confirmatory experiments of another year.

In 1926, out of doors, two rows of sunflowers were needle pricked in the very young flower head without introducing any crown-gall bacteria. Altogether 85 plants were used and of the same race as those previously used. The plants were 3 to 5 feet high when the heads were punctured and 7 to 10 feet high at the end of the season. The young flower heads of the west row (50 plants) received a half dozen punctures with a sharp needle. Those of the east row (35 plants) received one puncture only and in this case the procedure was varied by using a very blunt needle; i. e., one with the point filed off. I did this to see if it would not be possible to transplant bodily into the pith a fragment of the torus and thus break the continuity of the cyst and its subtending vascular bundles, but in this I did not succeed. From this east row in each case I obtained in the stem pith a continuous cyst with accompanying reversed vascular bundles. The length of these cysts varied, depending on the amount of growth made by the stems. All began in the receptacle. Many of these central pith stems (steles) were 3 to 4 feet long and some were longer. Two continuous pith cysts subtended all the way by the reversed vascular bundles (central steles) were 5 feet long. The west row developed many more cysts, corresponding to the greater number of pricks. In some of the stems there were as many cysts as pricks. With a very few exceptions, stem cysts were found in every plant examined, but only a much smaller percentage developed the floral anomalies (Pls. XXXVII and XLIII); i. e., 6 per cent in the east row (result of a single prick) and 12 per cent in the west row.

Some of the results of the above-mentioned experiments are shown on the plates, where especial attention is called to the appearance of the longitudinal sections.

The east row was punctured on July 6 and the west row on July 10.

The cysts and central steles are the result of transplants and subsequent intercalary growth, all of the wounded parts being young undeveloped tissues in which parts, as is well known, nearly all of the stem elongation takes place. If there was any downward growth, a matter still in

doubt, it must have been comparatively slight. The needle carries down surface and subsurface tissues much as a glove finger is inverted; hence all the tissues are bound to grow with a reversed polarity.

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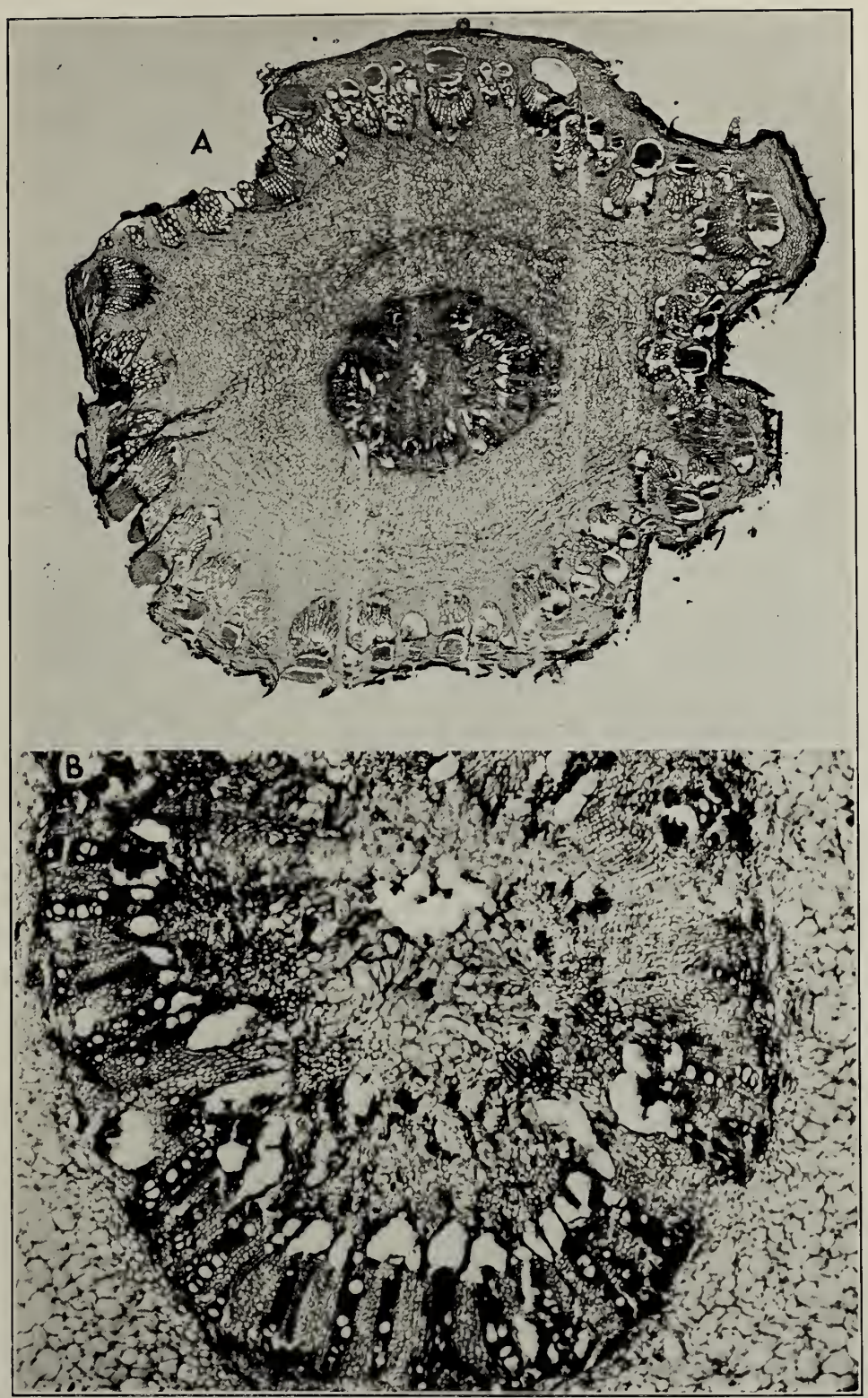
PLATES

PLATE I

A. Cross section of a sunflower stem 2.5 centimeters above the uppermost external tumor. One of the stem inoculations of 1924. Surface normal. Center of the pith occupied by a xylem-phloem cylinder, the order of which is reversed. Paraffine No. 1730. Coll. July 24, 1924. Planar. ×4.

B. Right side (¾ths) of the center of *A*, enlarged and rotated 90° showing more distinctly the vascular ring and the center occupied by disoriented parenchyma cells. This and a similar vascular cylinder in another stem, similarly inoculated (below the flower head) seem to have originated from the protoxylem region of the normal vascular cylinder.


A
B

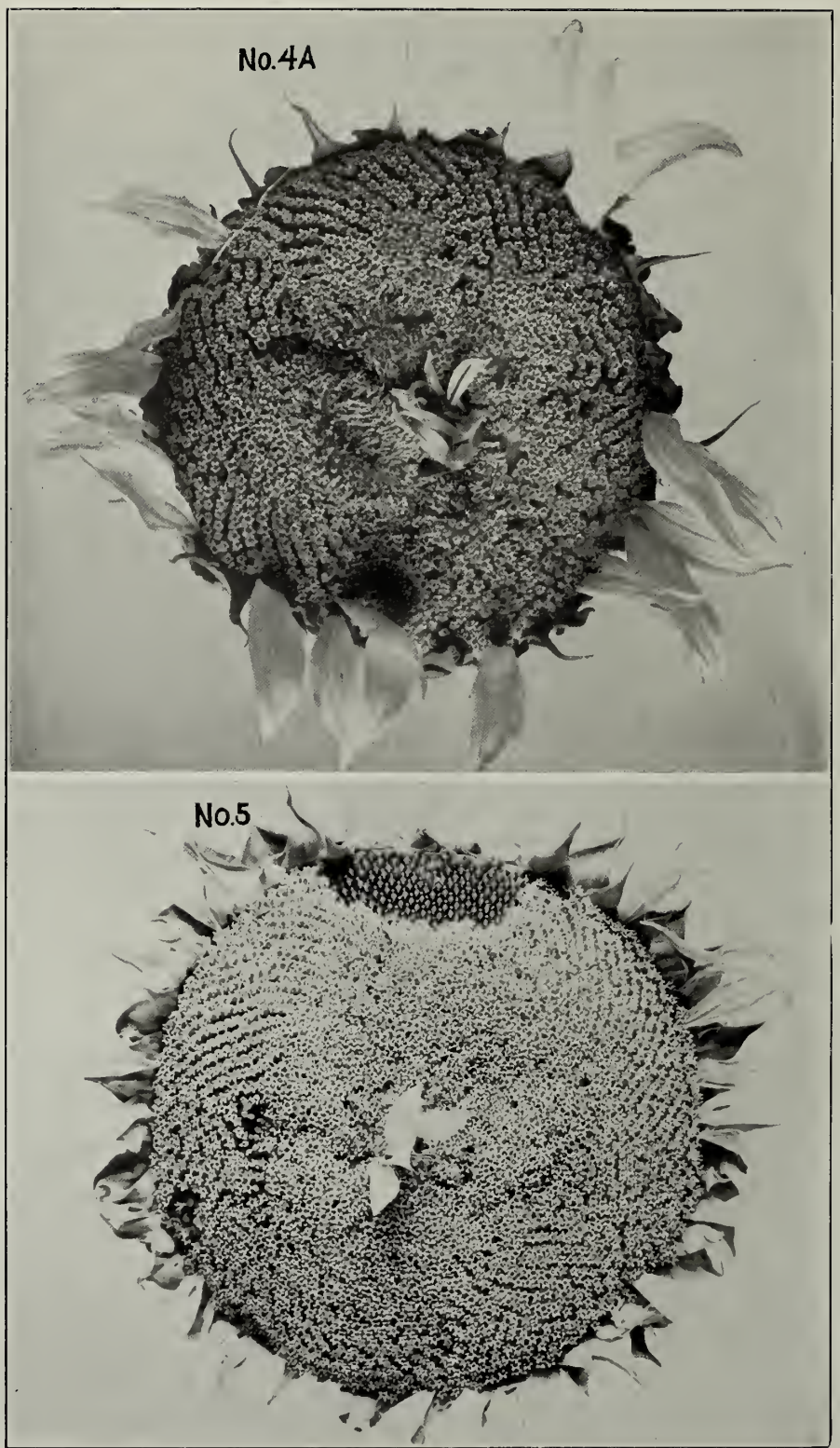

No.4A
No.5

PLATE II

No. 4A. Inoculated sunflower head showing green bracts and yellow rays developing among the tubular flowers. On dissecting the head three small tumors were found in its center under the ray flowers, one with two roots. Also on the stem there were four surface tumors and some unerupted ones, all on one side, and none in the pith. The last visible tumor was 15 inches below the head. Photographed July 21, 1925.

No. 5. Inoculated head showing ray flowers developing among the disk flowers. On dissection there was only a little crown-gall infection—hardly enough, it would seem, to account for the proliferation. Photographed July 21, 1925. Diameter of disk, about 9 inches.

PLATE III

No. 7. Inoculated head with central proliferation. None of the proliferated parts in this head showed complete supernumerary capitula, i. e., they had green bracts and ray flowers only on one side. There were 10 small tumors on the torus. The head was borne on a big stem the surface of which for a foot under the head bore a dozen rather small tumors. Inoculated June 18, 1925. Photographed July 23. About three-tenths natural size. Diameter of disk, 7 inches. There were 16 central ray flowers with subtending leafy green bracts from two distinct centers.

No. 8. There were 35 strap-shaped (ray) flowers in the center of this head with broad leafy bracts, but there were no completely separated supernumerary capitula, i. e., the green bracts and ray flowers were on one side only, making one-third, one-half, or two-thirds of a circle the rest of the way fused with other disk flowers, but here the disk flowers were conspicuously dwarfed and not yet open, while all the others up to them were in blossom. There were four centers of floral proliferation. There was tumor tissue in six places in the stem where it widened under the head. Photographed July 23, 1925. Diameter of the flower disk, 6½ inches. There were no surface tumors on stem of this plant and none on the torus.

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No.10A
No.12

PLATE IV

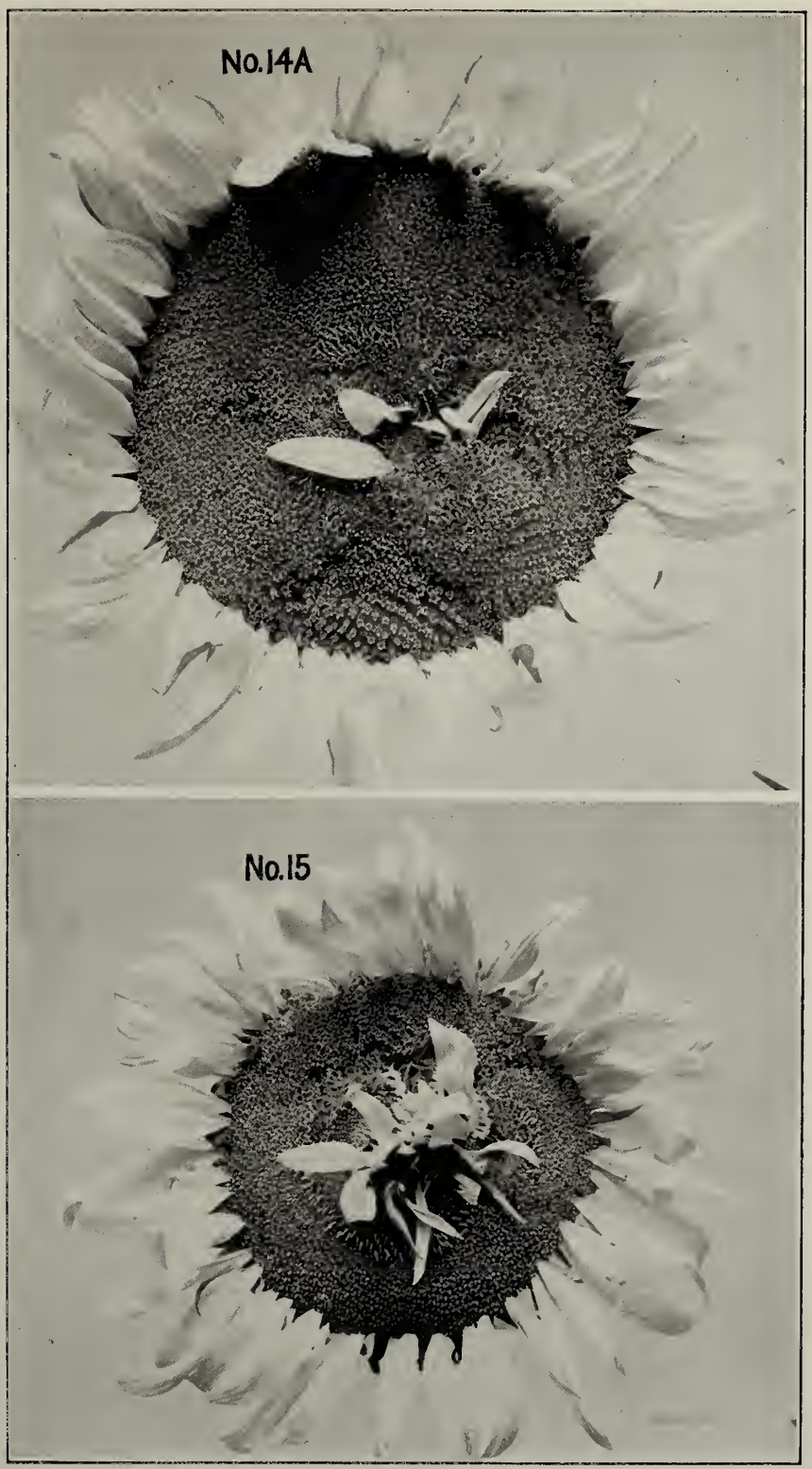
No. 10A. Inoculated head showing eight ray flowers (two groups either side of a crack) developing among the tubular flowers. The crown-gall proliferation was wholly in the stem under the head. Disk, 5 inches in diameter. Photographed July 24, 1925.

No. 12. Inoculated head showing copious proliferation There were 44 ray flowers in the center of this head, each with a leafy green bract like those on the periphery. These were in six groups arising from the torus. There were no tumors on the torus. In the pith of the stem immediately under the head there was an open cavity and several small closed cavities bearing greenish crown-gall tissue. The abnormal greenish spots continued downward in the pith 12 inches, i. e., to the lowest tumor, but not much beyond, only one of them that far. There were no tumors on the back of the head. Disk, 5 inches in diameter. Photographed July 24, 1925.

PLATE V

No. 14A. Inoculated head showing central proliferation. This head contained six secondary capitula, two of them short pediceled with the heads fused. (See 14B, Plate XVII.) There was tumor tissue with subtending vascular bundles in the stem just under the head. Externally there was a small stem tumor 18 inches below the head. There were no tumors on the back of the head and none on the torus. Photographed July 25, 1925. Circa three-tenths natural size.

No. 15. Inoculated head showing copious floral proliferation from the center. There were 37 central ray flowers (20 not open). There was no split in the torus. One of the four secondary capitula (all of which were sessile) was nearly complete, i. e., it had broad green leafy bracts and yellow ray flowers three-fourths of the way around. The other one-fourth of the way it was fused into the ordinary disk flowers. There were two green tumor strands in the center of the pith immediately under the head. One of these extended down the pith 12 inches. There were no surface tumors on the stem and none on the torus. Photographed July 25, 1925. Circa three-tenths natural size.


No.14A
No.15


No.16
No.17

PLATE VI

No. 16. Inoculated head showing central proliferation. There were seven ray flowers in the center and tumors and anomalous vascular strands in the pith downward for a distance of 19 inches. Several of these tumors were in the pith cavity close under the head. There were unruptured tumors in the cortex 9 inches down and ruptured ones in a leaf axil 3 inches down. No tumors on the torus. Diameter of disk, 4½ by 5 inches. Photographed July 25, 1925.

No. 17. Copious proliferation of ray flowers in the center of an inoculated head which was cracked open irregularly. It showed no complete subcapitula. No tumors on the torus, but tumor tissue in the stem immediately under the head and farther down the stem, i. e., 7 inches down and in a leaf axil 16 inches down. Diameter of the disk, the largest way, about 9 inches. Plant tall, stem large. No tumors on the back of the flower head. Photographed July 25, 1925.

PLATE VII

No. 19A. Inoculated sunflower head showing 33 central ray flowers developed along both sides of a central crack extending nearly across the head. There were no tumors on the torus. The stem under this head had two opposing longitudinal deep grooves cutting the pith nearly to the center. These grooves were subtended by groups of vascular bundles and lined with white hairs. There were small crown galls on the surface of the stem 4 to 9 inches down and larger ones 16 to 20 inches down. The petioles, 12 and 16 inches down, also bore small tumors. The abnormal phenomena in the stem continued all the way down to the large tumors and through the whole of that region (to 20 inches down) but not beyond. Photographed July 27, 1925.

No. 19B. Same as the preceding but with the anomalous ray flowers removed to show the underlying abnormal green bracts. On dissection *X* proved to be a perfect sessile small subcapitulum. Disk, about 5 inches in diameter.


No.19A
No.19B
X

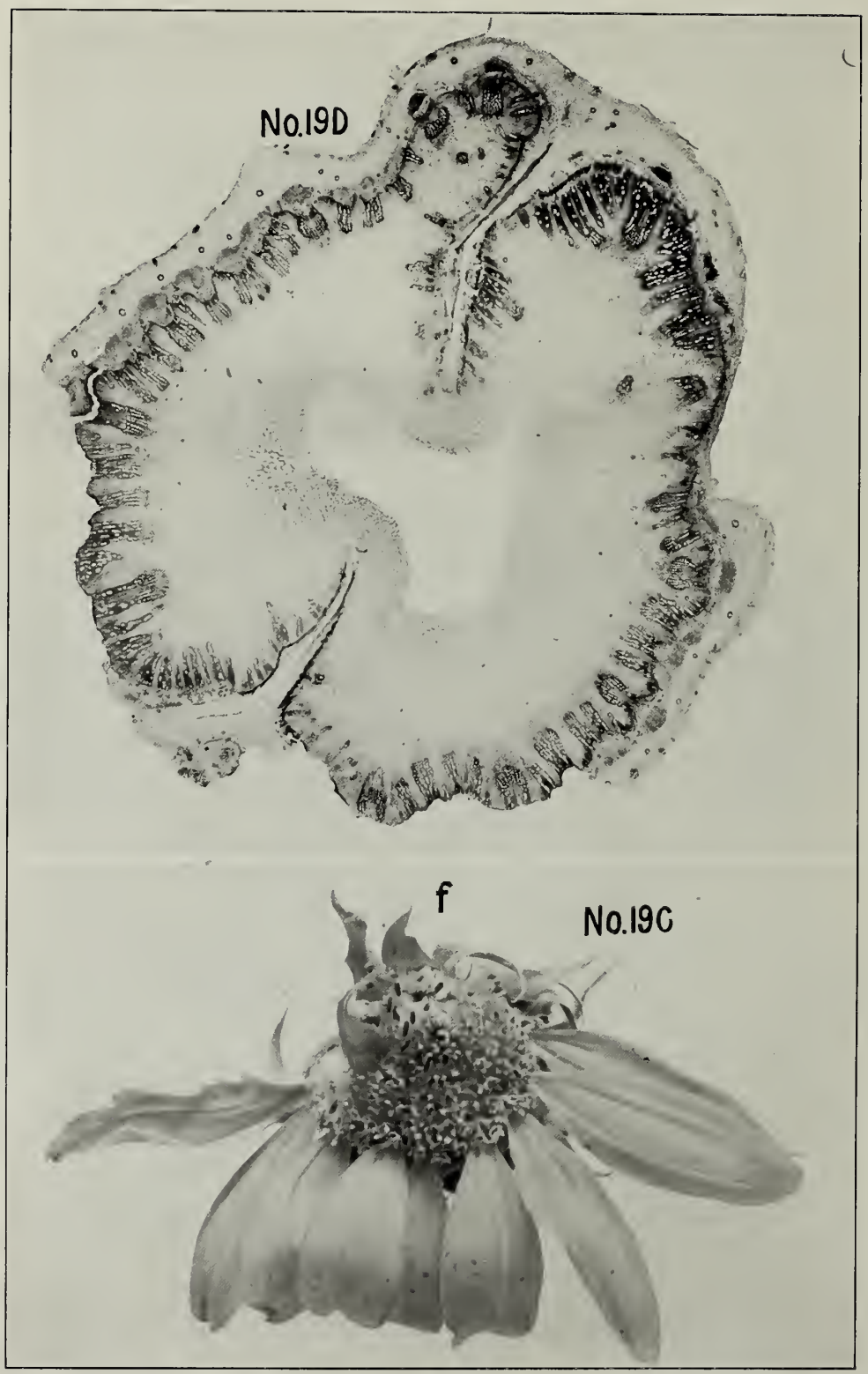

No.19D
f
No.19C

PLATE VIII

No. 19C. The small supernumerary capitulum referred to under 19B (Pl. VII). The upper rays at *f* were removed before it was discovered.

No. 19D. Cross section of stem of No. 19A, 14 inches below the head, showing two long slits subtended by vascular bundles (except in inner part of right side of lower slit) and involving pith, xylem-phloem, and cortex. The white center is dead pith which did not hold the stain—methylene blue and acid fuchsin. Planar. Circa×4.

PLATE IX

No. 1A. Center of an inoculated head full of abnormal green bracts like those occurring normally on the periphery of the flower head. They subtended central ray flowers which were just beginning to develop. Most of the latter were still folded and small. There were three supernumerary sessile capitula in the center of this head. This and the following photo were made early so that the central ray flowers might not obscure the large green bracts. Inoculated June 18, 1925. Photographed July 18. Centrally there was crown-gall tissue in the stem under the torus, and there was one tumor on the torus. Disk, about 8 inches in diameter.

No. 2. Inoculated flower head showing central proliferation (ray flowers and leafy bracts, like the marginal ones). The central ray flowers are only just beginning to expand. On dissection a little crown-gall tissue was found in the stem immediately under the head, but none in the head. In this head there were four fully formed sessile subcapitula.


No.1A
No.2

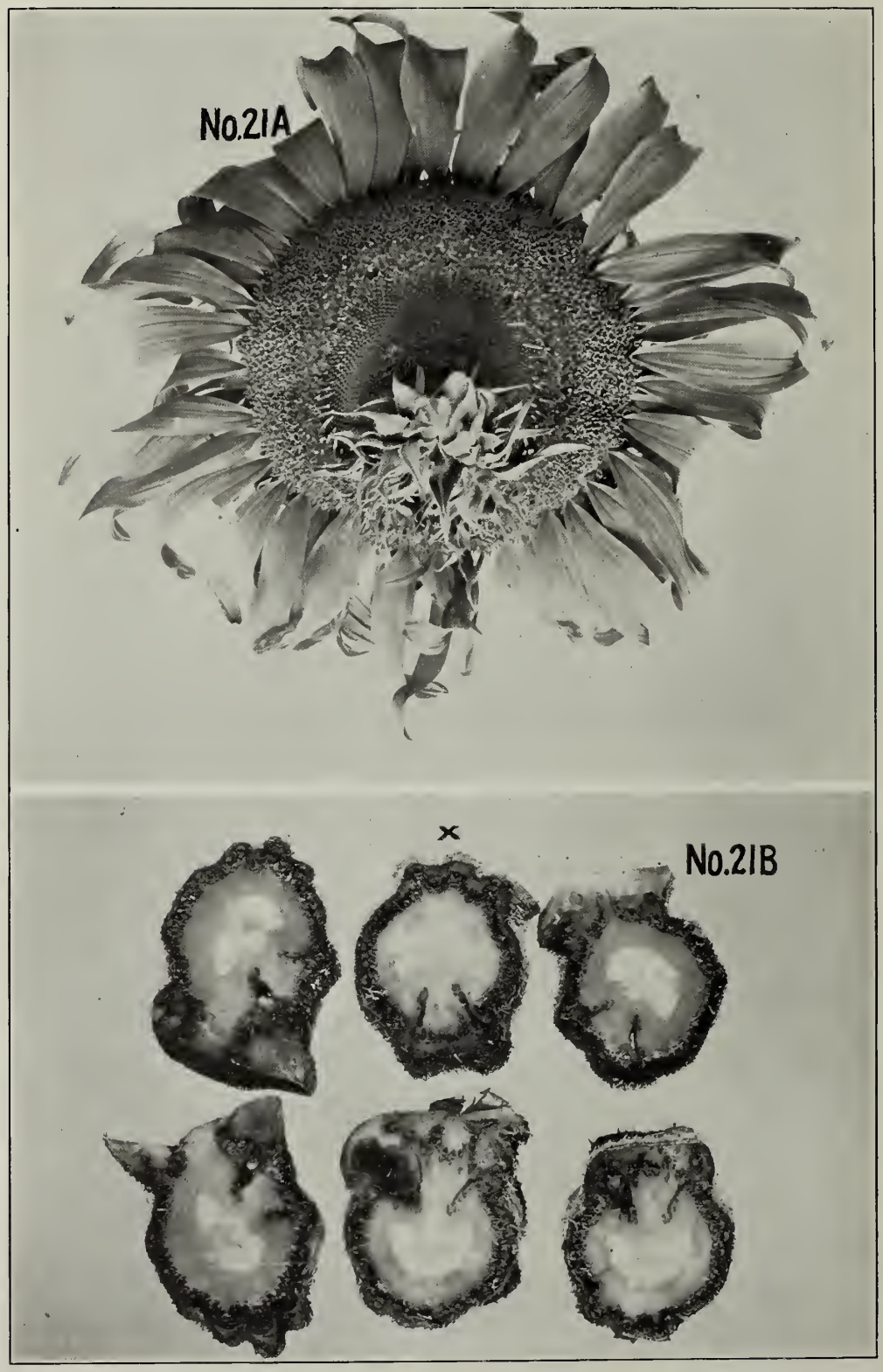

No.21A
No.21B

PLATE X

No. 21A. Like Nos. 1A and 2 (Pl. IX) green bracts well developed, but the yellow ray flowers in the proliferous part not yet open. There were no tumors on the torus and no perfect supernumerary capitula. In the pith of the stem where it widened just under the head there were two cysts lined by a membrane covered with soft white hairs like those on the torus. These cysts were continuous down the stem pith 7 inches. There was then 3½ inches of bulging but unruptured tumors on one side, occluding and expanding the cyst cavities. Below this for 5 inches the stem was normal externally but with two slits in the pith lined with a hairy membrane. Six inches below this cut was an axillary tumor one-half inch in diameter and 5 inches lower another of the same size. Undoubtedly the cyst cavities continued down to the lowest tumor (26½ inches below the head). Disk, 6 inches in diameter. Photographed July 28, 1925.

No. 21B. Cross sections of stem of 21A at various levels all above 21C (Pl. XI), showing open and tumor occluded cysts. Total thickness of these pieces, 2½ inches. An enlarged section of *x* is shown in 21BB (Pl. XI).

PLATE XI

No. 21BB. Enlarged cross section of 21B (Pl. X) showing pith cavities subtended by vascular bundles. Sometimes the surrounding vascular bundles have a central core of xylem entirely surrounded by phloem, as at *x*, for an enlargement of which see Plate XXXIII, *A*. These cavities, lined with soft white hairs like those of the torus, extended down the stem a distance of 16 inches. For tumors on their walls see 21B (Pl. X) and 21C. Observe here also, as in Plate VIII, 19D, irregular arrangement of the vascular bundles subtending the cysts. Acid fuchsin stain. Planar enlargement. ×3.7, circa.

No. 21C. Longitudinal section of stem 7 to 10½ inches below the inoculated flower head showing three tumors filling and stretching the cyst. Actual length of this piece, 3½ inches. Below this point as far as examined (5 inches) the cysts continued as closed pith cavities lined with a hairy membrane.

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No.22A
No.22B

PLATE XII

No. 22A. Plant 11 feet high with a big stem but badly attacked by tumors in the upper part, hence the dwarfing of the head. The external tumors (numerous and large) began 11 inches below the head and continued down the stem an additional 18 inches. (Pl. XIII.) The vascular bundles in the pith continued down the stem 28 inches, i. e., almost as far as the tumors, but not beyond them. Three sessile supernumerary capitula were present. There were no tumors on the torus, but under it in the pith were hairy cavities occasionally filled with tumor tissue and always subtended by vascular bundles. These cavities started in the torus. The hairy lining was very conspicuous. Disk, 4 inches, circa. Photographed July 28, 1925.

Compare with Cockerell's figure "Chimera in head of sunflower," The Journal of Heredity, January, 1925 (frontispiece).

No. 22B. Two of the three supernumerary capitula from 22A. The right-hand one had not yet developed its ray flowers.

PLATE XIII

No. 22D. Part of the stem of 22A (Pl. XII) bearing large surface tumors. Some of these originated from the cambium. Natural size. The hairy-lined cysts continued through this piece into 22C.

No. 22C. Stem immediately under No. 22D. Slightly less than one-half natural size. At *X* its tumors are still unruptured.

In Berlin in 1925, in Doctor Blumenthal's laboratory at the Charity Hospital, I saw tumors on sunflowers (quite like these) produced with bacteria cultivated from a human breast cancer, which organism also produced metastasizing malignant tumors in rats.


22D
22C
X


No.11A
No.11B

PLATE XIV

No. 11A. Inoculated sunflower head showing anomalous floral proliferation. There were 35 central ray flowers with big green bracts like those shown on Plates IX, X, and XII. These were from seven centers of proliferation. Under the head in the pith and for many inches down the stem there was a hairy cavity with tumors here and there on its walls, the tumors beginning close under the head. This pith cavity was subtended by vascular bundles and extended as far down the stem as the last tumor (18 inches below the head). There were no tumors on the torus. Disk, 7.5 inches.

No. 11B. Longitudinal section of stem cavity (cyst) 10 inches below No. 11A, showing hairs and two small tumors. One side of the cyst wall is smooth. × 6.7, circa.

PLATE XV

No. 9. Inoculated proliferous head. Stem also furrowed downward 18 inches with small superficial tumors on its upper 14 inches, but no tumors on the torus and no conspicuous ones in the pith immediately under it. In this case the edges of the split in the head are bordered all the way (10 inches) with big green bracts and ray flowers. There are also two side splits not so wide open showing the same phenomena.

No. 13. A case in which inoculation and complete tearing apart did not cause floral proliferation. There are small tumors on both parts of the split stem, but none in the pith, and only one on the torus (margin at X). Probably the head was too old when inoculated or grew too slowly. Photographed July 24, 1925.


No.9
X
No.13

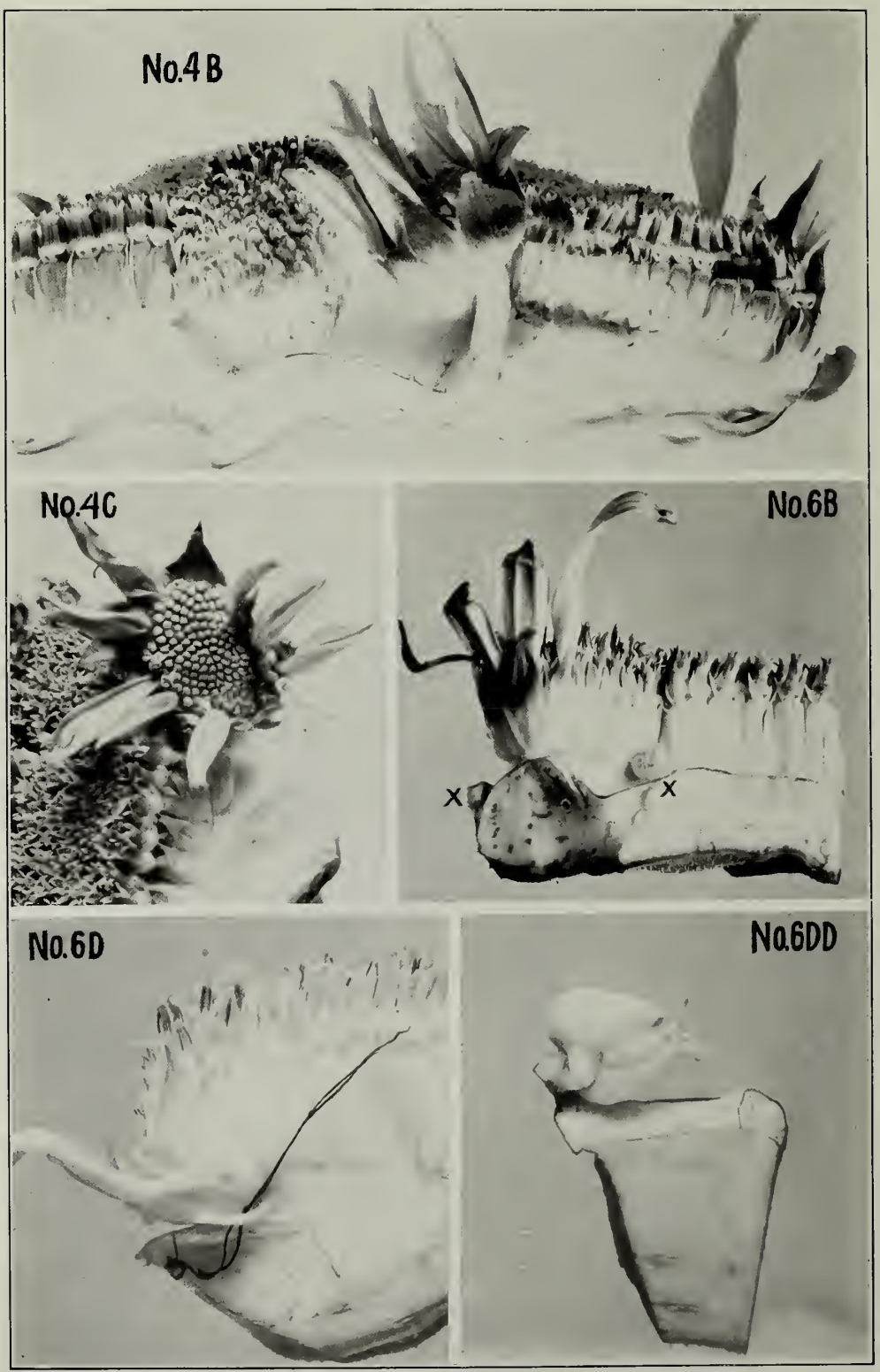

No.4B
No.4C
No.6B
X
X
No.6D
No.6DD

PLATE XVI

No. 4B. One half of the inoculated head No. 4A (Pl. II), showing a wholly independent small stalked capitulum provided with broad green bracts, a dozen yellow-ray flowers and about 100 disk flowers. Pedicel cylindrical, smooth, pure white, seven-eighths inch long, one-fourth inch in diameter. It is a perfect little head developed in the center of another head, as a rose flower sometimes develops a second rose out of itself. It differs from those of Nos. 1A and 2 (Pl. IX) only in being pediceled. The clear space around this head is scar-tissue. For a top view see No. 4C. The other half of this disk bore three crown galls. Three-fifths natural size, circa.

No. 4C. Top view of 4B, disk flowers less advanced than in the adjoining normal part. Inoculated June 18. Photographed July 21, 1925.

No. 6B. Center of a head showing abnormal proliferation (green bracts and five yellow rays), with torus tumors at X. This is a sessile imperfect supernumerary capitulum. There was a big tumor on the stem 2 feet below this head. Diameter of disk, 7 inches. Inoculated June 18. Photographed July 23.

No. 6D. Another proliferation from No. 6, with a root growing from the torus near it. Sections of this root were cut and examined.

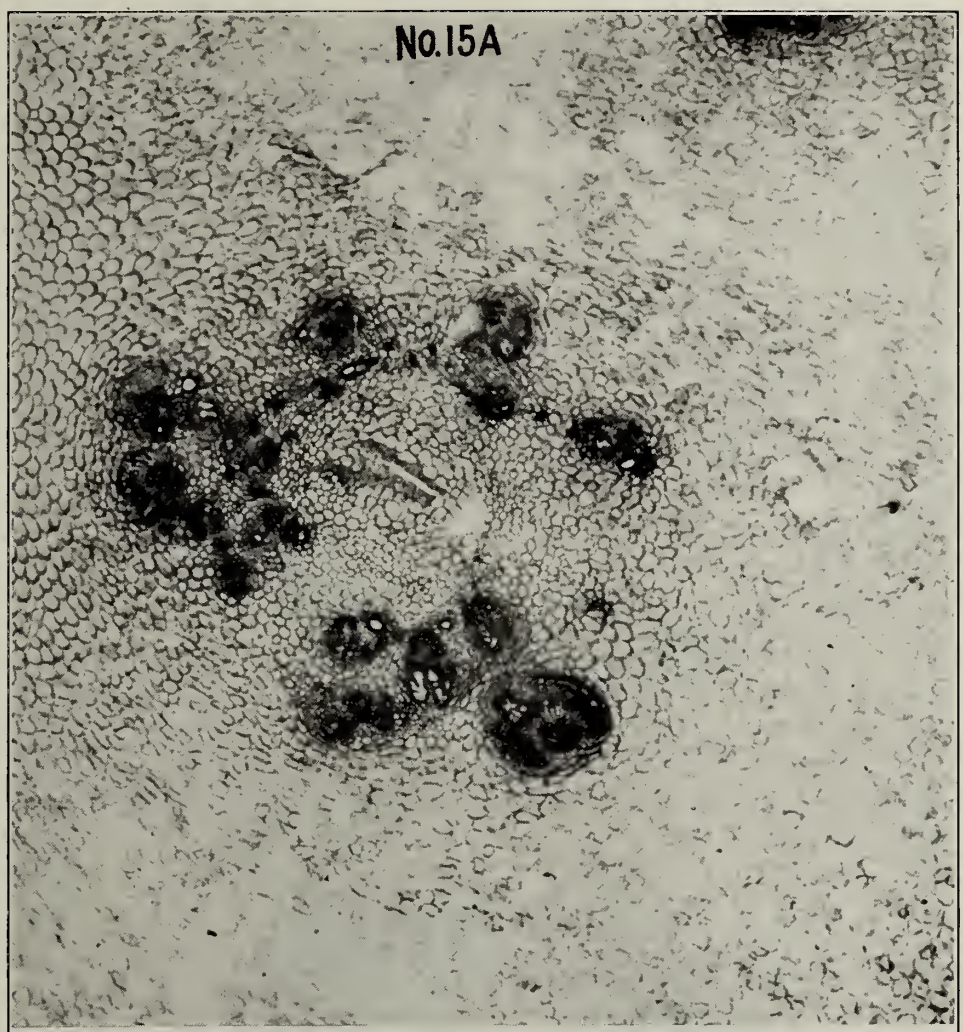
No. 6DD. A third short pediceled supernumerary capitulum not yet in blossom. The smooth surface below is healed tissue of the torus. The capitulum is white because it was buried in the head and not visible until the latter was dissected. There were six centers of floral proliferation in this head.

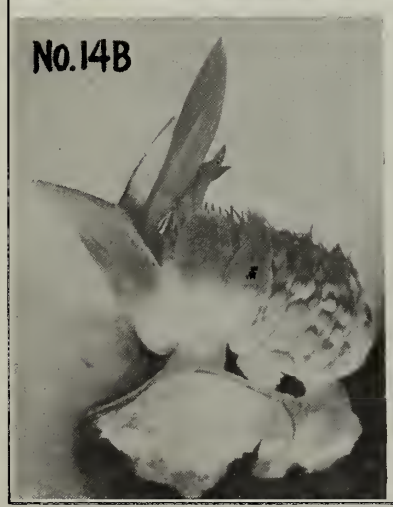
PLATE XVII

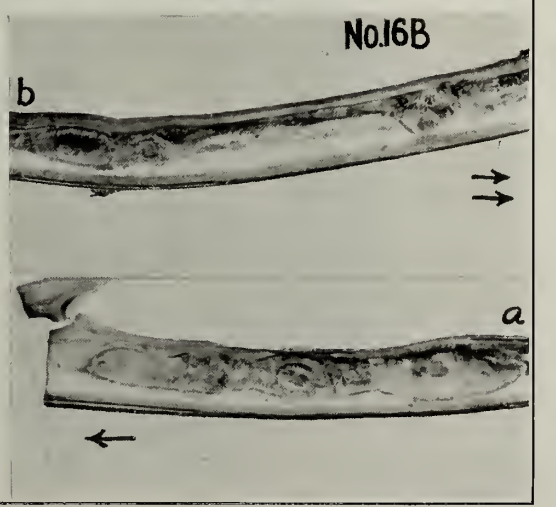
No. 14B. Two stalked supernumerary capitula from the center of a sunflower disk (Pl. V), heads fused, one head showing at the left large green bracts and yellow ray flowers, the other head having only unopened tubular flowers. Photographed July 25. Three-fourths natural size, circa.

No. 15A. A small cyst in the stem pith of No. 15 (Pl. V), surrounded by vascular bundles. Sectioned directly from Carnoy. Planar enlargement without use of the microscope. × 27, circa.

No. 16B. Longitudinal section of the stem of No. 16 cut 3 to 10 inches below the inoculated head. Pith containing a cyst which is full of tumors. Stem bulged in places by growth of these tumors. The double arrow points toward the base of the stem and the single arrow points toward the flower head. *a* joins on to *b*.


No.15A


No.14B


No.16B
b
a

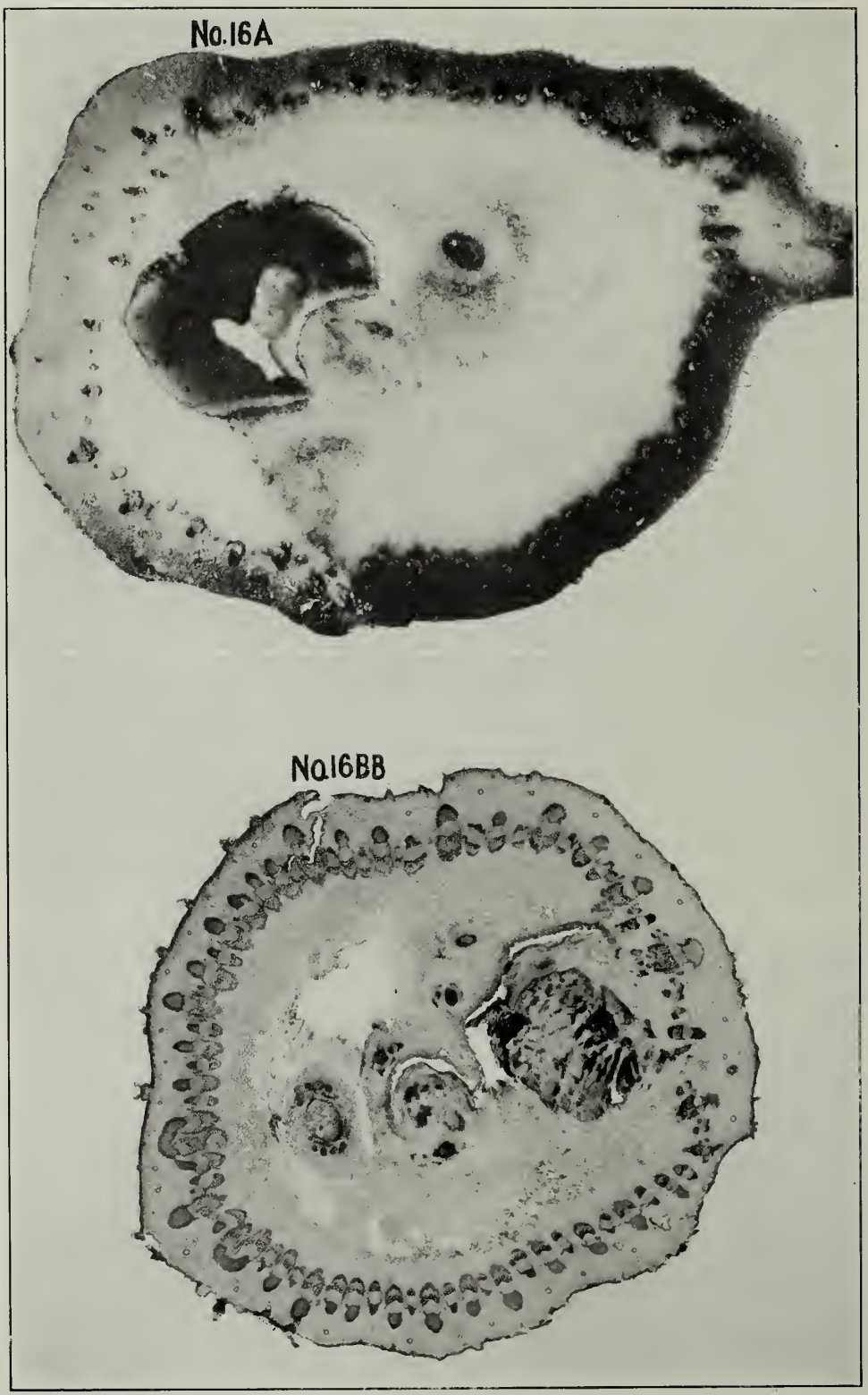

No.16A
No16BB

PLATE XVIII

No. 16A. Cross section of stem close under the inoculated head of No. 16 (Pl. VI), showing three cysts in the pith, two bearing tumors. Frequently these tumors filled the cavity and greatly expanded it, as in No. 16B (Pl. XVII) and No. 16BB. Photographed July 25, 1925. Circa × 3.2.

No. 16BB. Cross section of stem cut 2 inches below the inoculated head, i. e., below 16A and above 16B. (Pl. XVII.) This shows three cysts in the pith, each bearing a tumor and subtended somewhat irregularly by vascular bundles. Stained with pyronine and methyl violet. Planar. Circa × 5.

PLATE XIX

No. 16(C). Cross section of central part of stem cut below 16B (Pl. XVII), i. e., 12 to 16 inches below the inoculated head showing two tumor-bearing cysts subtended by vascular bundles developed in the pith. The normal vascular bundles may be seen on either side at the extreme top. The lighter areas are pith cells which have not stained well, i. e., pith that was dying. Here, as in all the other cases, the xylem-phloem of the subtending vascular bundles is reversed, the phloem pointing toward the cyst cavity. Cut from Carnoy, stained with methylene blue and acid fuchsin and photographed with an ordinary camera using a Zeiss planar lens. Circa × 17.

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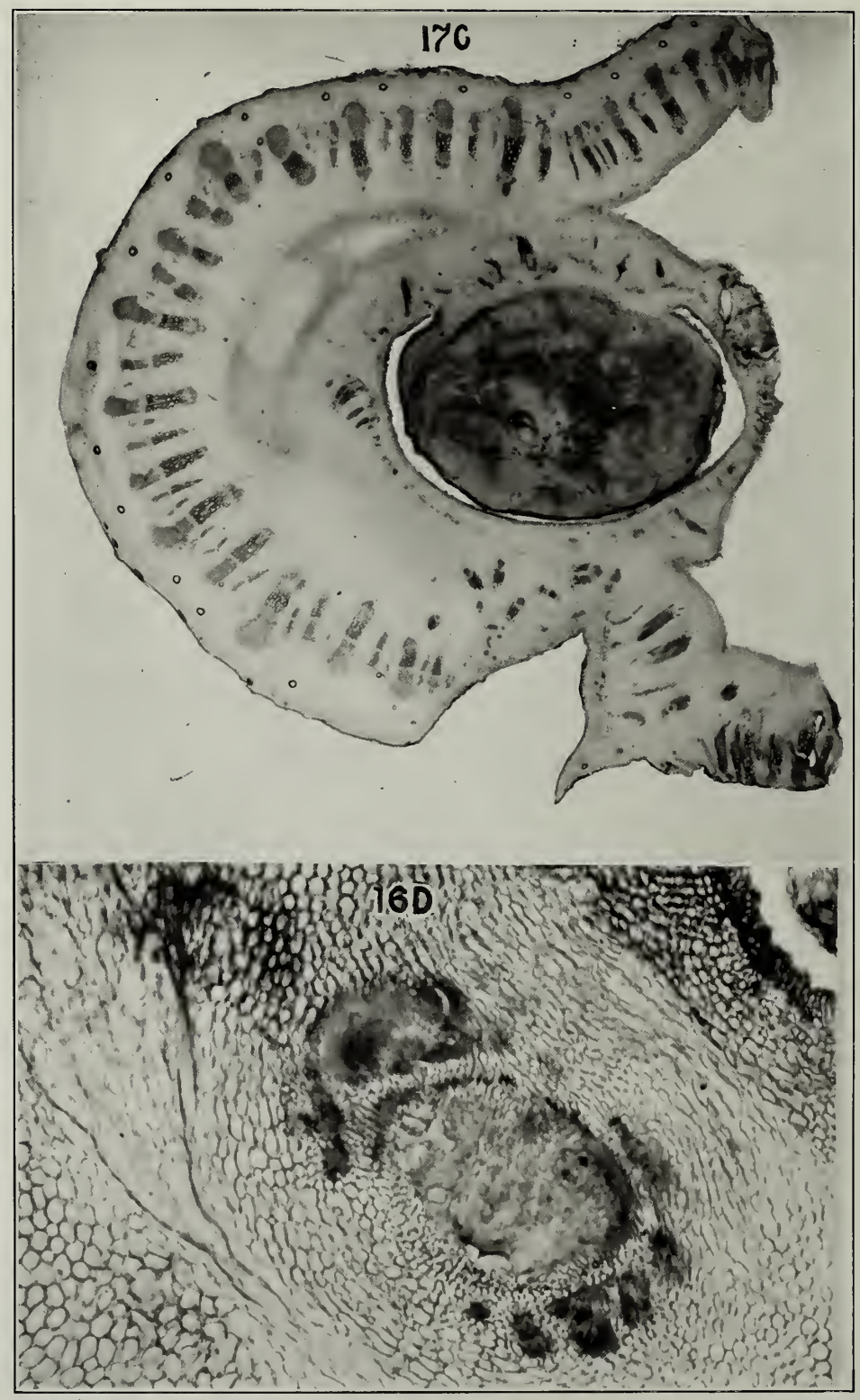

17C
16D

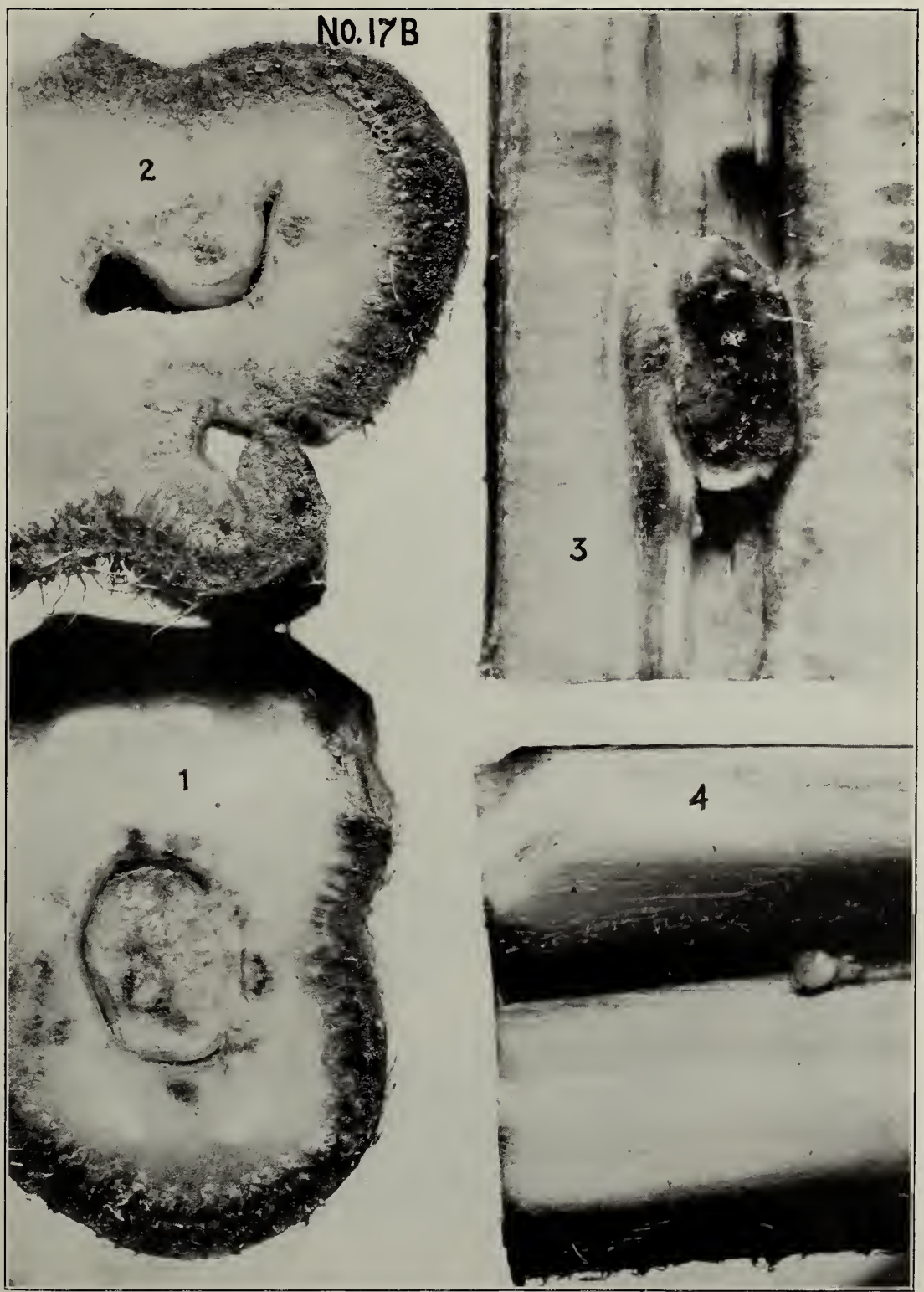
PLATE XX

No. 16D. Left hand tumor of 16BB (Pl. XVIII), with extreme edge of the middle cyst in the upper right corner. Acid fuchsin and methylene blue. Planar × 35.

No. 17C. Cross section of stem near 17B1 (Pl. XXI) showing the cyst subtended by vascular bundles and bearing a broad pedicled tumor on its wall. The tumor is full of twisted vascular bundles containing pitted vessels which have no connection with the normal vascular bundles of the stem. This section is about 8 inches below the flower head. Here also the walls of the cavity bear scattering hairs. Cut direct from Carnoy. Stained with pyronine and methyl violet. Planar. Circa × 4.

PLATE XXI

No. 17B. Cyst and cyst tumors at different levels in the stem pith of No. 17. Figure 1 was cut 8 inches down the stem; Figure 3 (longitudinal) was 14 inches down; and Figure 4 was still farther down. Figure 2 was cut below Figure 1. Above Figure 1 was a cyst cavity extending all the way to the flower head. This, as usual, bore soft white hairs like those on the torus. The hairs are also here at each level but less conspicuous and not very abundant. They are best seen in Figure 4. Circa × 3. For an enlarged cross section made not far from No. 1, see Plate XXII.


No. 17B
2
3
1
4


No.17E
P
P
X

PLATE XXII

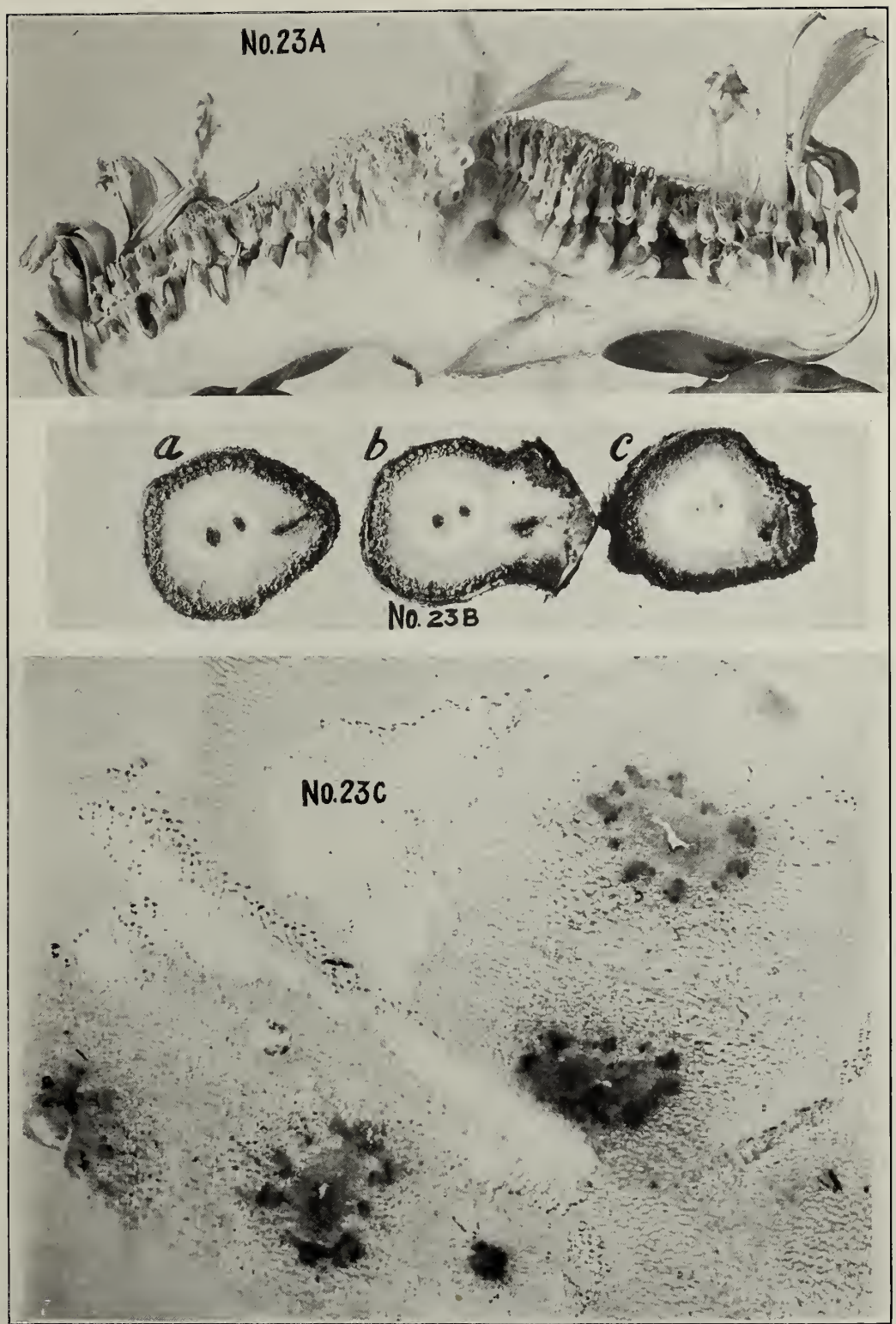
No. 17E. Same tumor as 17C (Pl. XX), but from another section, further enlarged to show the structure of the cyst wall. Stained with acid fuchsin. Xylem-phloem reversed. Normal vascular bundles at *X*. P, P is pith. Circa ×17.

PLATE XXIII

No. 23A. Cross section of an inoculated head showing a floral proliferation in its center (yellow rays and broad green bracts). This was an imperfect sessile supernumerary capitulum with three ray flowers. In the center of the torus there is a hairy small opening (healed needle wound). There were four of these in the receptacle, each lined with a hairy membrane. No tumors on the torus or on the back of the head.

No. 23B. Cross sections of stem of 23A at various levels showing three cysts with subtending xylem-phloem bundles in the pith; *a*, near the upper end of the stem, i. e., within 6 inches of the inoculated head; *c*, 16 inches below the head; *b*, midway between *a* and *c*. Enlarged slightly.

No. 23C. A free-hand cross section, water mount, made near the flower head, i. e., above 23B *a*, showing a portion of the pith containing interrupted vascular bundles subtending six cysts. Two inches farther down there were four cysts. They were greenish. In all cases the phloem faced the cavity. Planar. ×10, circa.


No.23A
a
b
c
No. 23B
No.23C

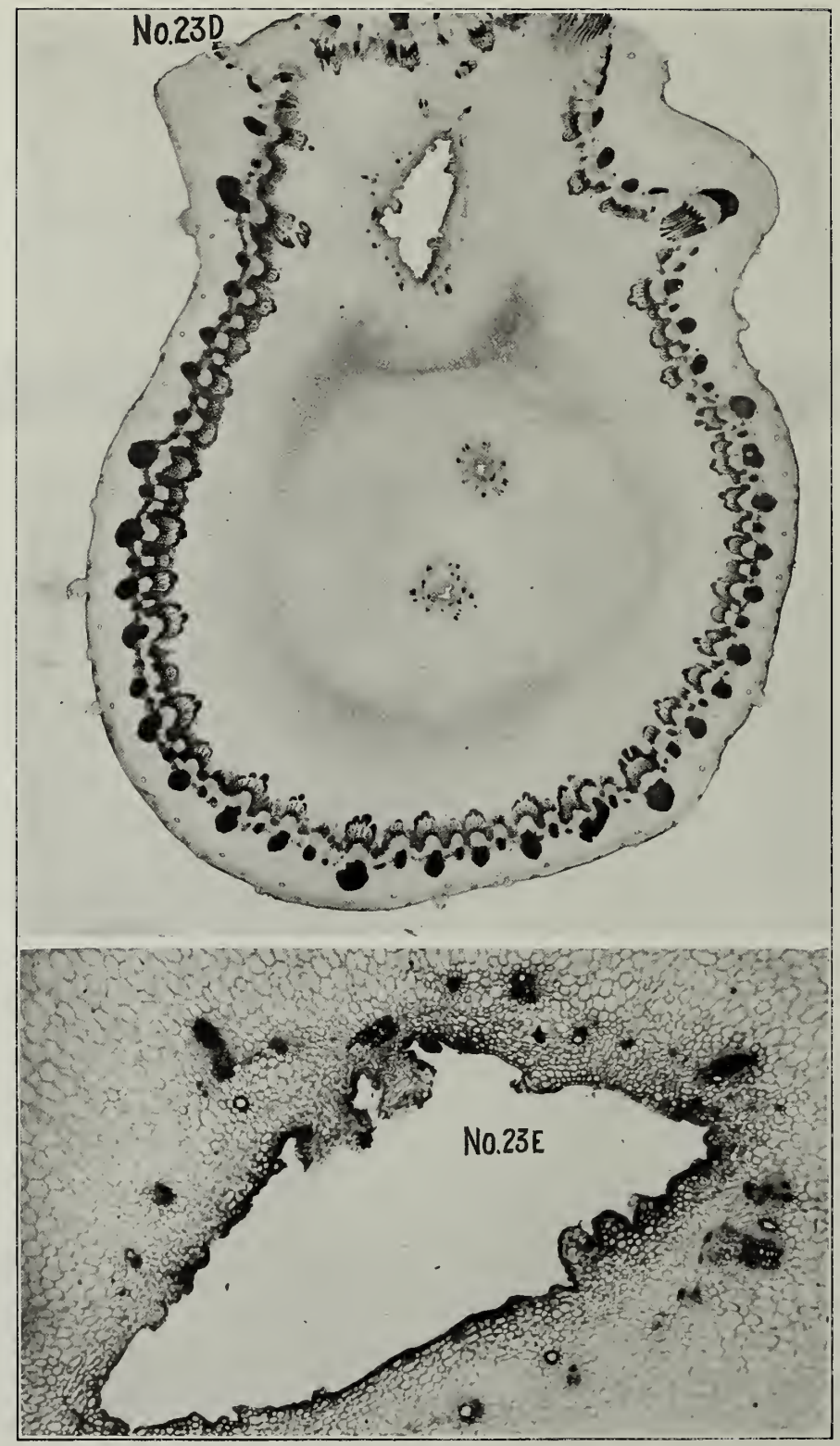

No.23D
No.23E

PLATE XXIV

No. 23D. Cross section of stem 10 inches below the inoculated flower head, i. e., from 23B sub. *b* (Pl. XXIII), showing three cysts in the pith surrounded by very regularly oriented vascular bundles. These were continuous in the pith for a distance of 16 inches from the head; 8 inches farther down was a small surface tumor not connected with any pith strand. As usual the xylem-phloem is reversed. Cut without embedding. Stained with pyronine and methyl violet. Planar. ×3, circa.

No. 23E. The larger cyst of 23D showing small tumors and glandular trichomes on its fine-celled wall. Stained with methylene blue. Planar. ×27, circa.

PLATE XXV

No. 10B. Section of stem of 10A (Pl. IV) just under the inoculated proliferous head. This section shows two cavities in the pith (cysts) lined with a membrane bearing numerous soft white hairs like those on the torus. Like all of these pith cavities they are probably invaginations of the young meristematic torus. The upper one at *X* bears small tumors. Each cyst is subtended by vascular bundles which have developed in the pith. The crown galls were wholly in the interior of the stem, under the head. Photographed July 24. ×6.7, circa.

No. 10C and 10CC. Longitudinal sections of the stem of No. 10 cut 6 to 8 inches below 10B, showing cyst walls bearing tumors and numerous soft white hairs. Photographed July 24.


No.10C
No.10B
X
No.10CC

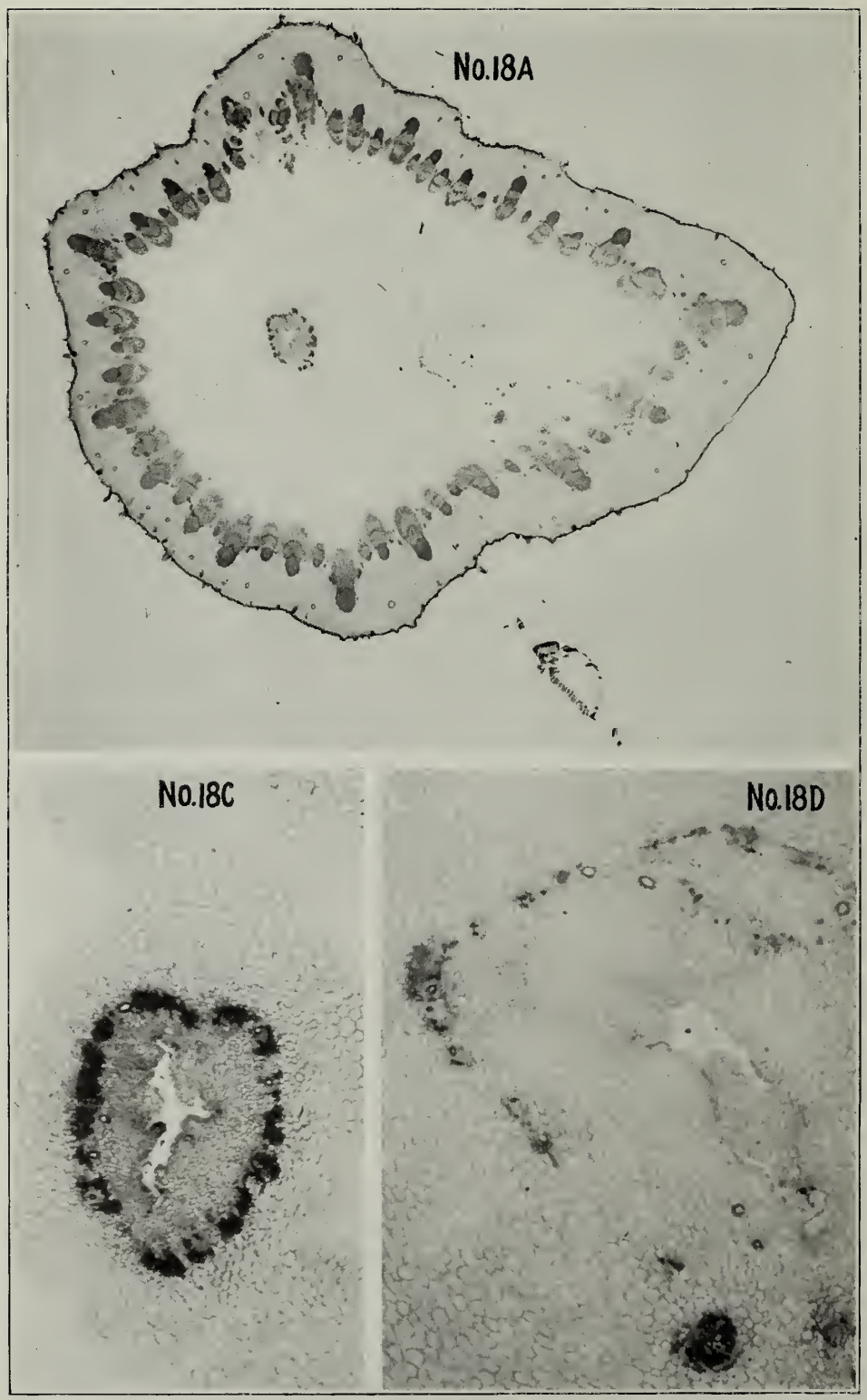

No.18A
No.18C
No.18D

PLATE XXVI

No. 13A. Cross section of stem a few inches under the inoculated proliferous head, showing three cyst cavities in the pith, all of them bearing small tumors on their walls. The third cyst is on the upper left side near the vascular ring. One of these cysts extended down the stem a distance of 18 inches. These cavities are lined with a membrane bearing hairs. Stem small, no surface tumors and only one ray flower and leafy bract in the center of the disk flowers. There were three tiny cavities in the torus lined with white hairs and continuing down the stem (healed needle thrusts). No tumors on the torus, none on the back of the head.

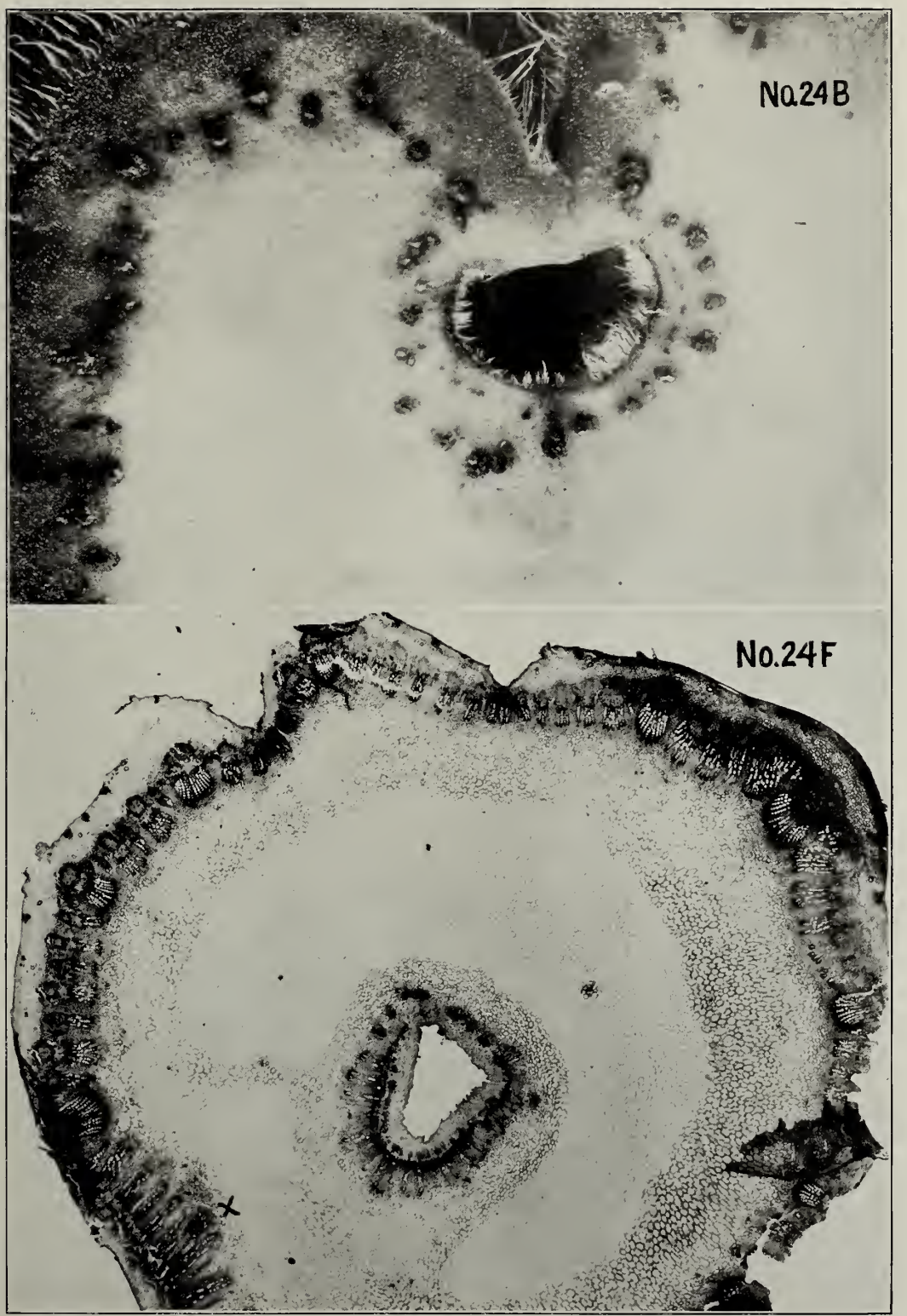
No. 18C. Left-hand cyst of 18A in center of pith, enlarged to show details of structure especially the fine-celled tissue inside the dark ring which consists of vascular bundles. Cut direct from Carnoy. Planar enlargement without use of the microscope. Circa ×27.

No. 18D. Right-hand cyst of 18A showing the small tumor and the subtending vascular bundles embedded in the pith. Planar. Circa ×27.

PLATE XXVII

No. 24B. Uppermost piece of stem under an inoculated proliferous head, showing a cyst in the pith, lined with thickset hairs (for enlargement of which see Pl. XXXVI) and subtended by interrupted vascular bundles. Further down (as growth continued) this cavity came to the surface as a deep sinus, while above it extended through the pith of the head to the torus as a cylindrical hole (diameter, one-fourth inch) lined with white hairs. Fifteen inches down the stem the surface groove became again a closed pith cavity (greenish) with a hairy lining membrane and here the subtending vascular bundles were fused into a compact cylinder of xylem-phloem which continued down the stem for several inches (more than 6) with occasional tumors on the walls. There were no tumors on the back of the head or on the upper 21 inches of the stem; 2 feet lower (45 inches from the head) there were unruptured tumors on one side of the stem.

No. 24F. Cross section of stem 16 inches below 24B showing a cyst in the center of the pith surrounded by a compact reversed vascular cylinder. Sectioned from fixed material preserved in alcohol. For a detail at *X* (phloem buried in xylem) see Plates XXXIV and XXXV. Stained with methylene blue and acid fuchsin. Planar. ×6, circa.



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No.24D
t
x
+
X

PLATE XXVIII

No. 24D. Enlargement of 24F (center of stem) showing details of the complete xylem-phloem cylinder around the cyst on the walls of which are hairs and incipient tumors. The dark stained patches in the inner fine-celled tissue are islands of hard bast. *T*, tumor; *XXX*, glandular hairs. The cells surrounding this anomalous tissue are normal pith cells. The lining membrane of this cyst (left side) is shown further enlarged on Plate XXXIII. Planar ×32, circa.

PLATE XXIX

Ray flowers among the tubular flowers. In the lower head and also on Plate XXX observe retarded development of the tubular flowers in the proliferous part.

This plate and the next three were made from the second sunflower crop of 1925 (Series III) wounded in the young flower head by needle pricks without inoculation.

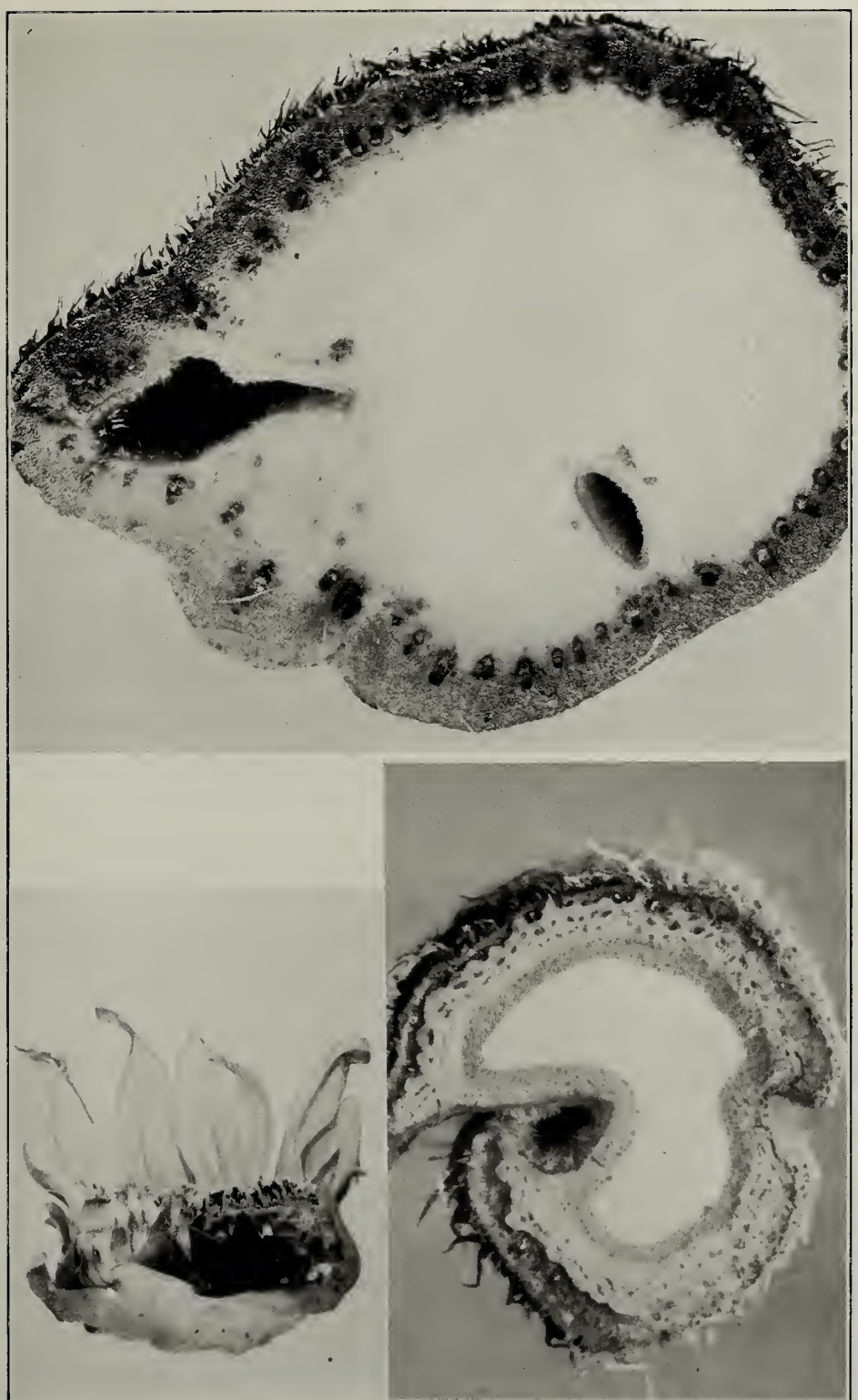
PLATE XXX

Dissections of three proliferous flower heads. One shows a linear green bract as well as a broad green bract and one ray flower among the tubular flowers. Needle pricks only.

PLATE XXXI

Cross section of two stems showing cysts with hairy walls. Also the cross section of a flower head showing broad green bracts developed among the tubular flowers. Needle pricks only.

38



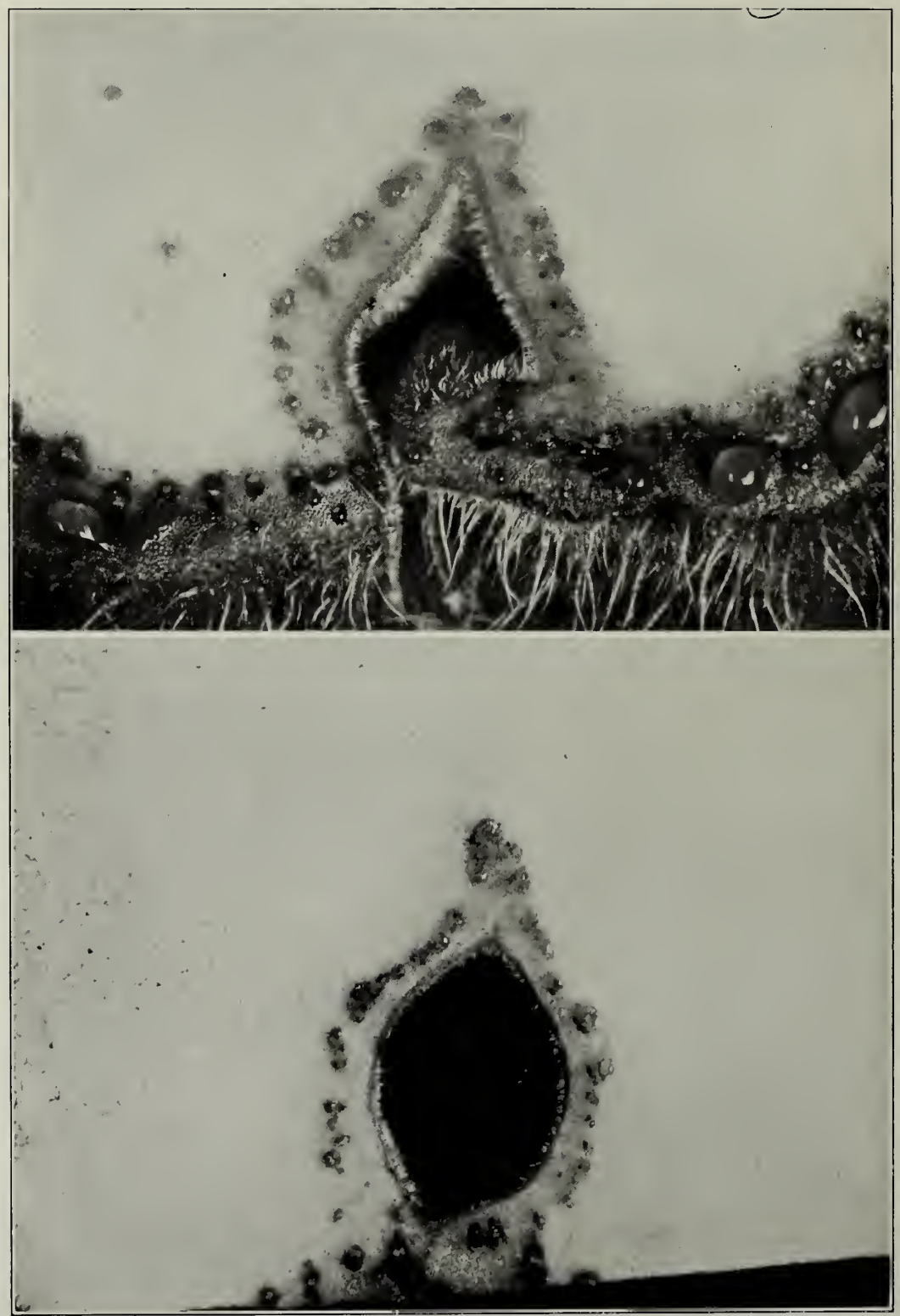


PLATE XXXII

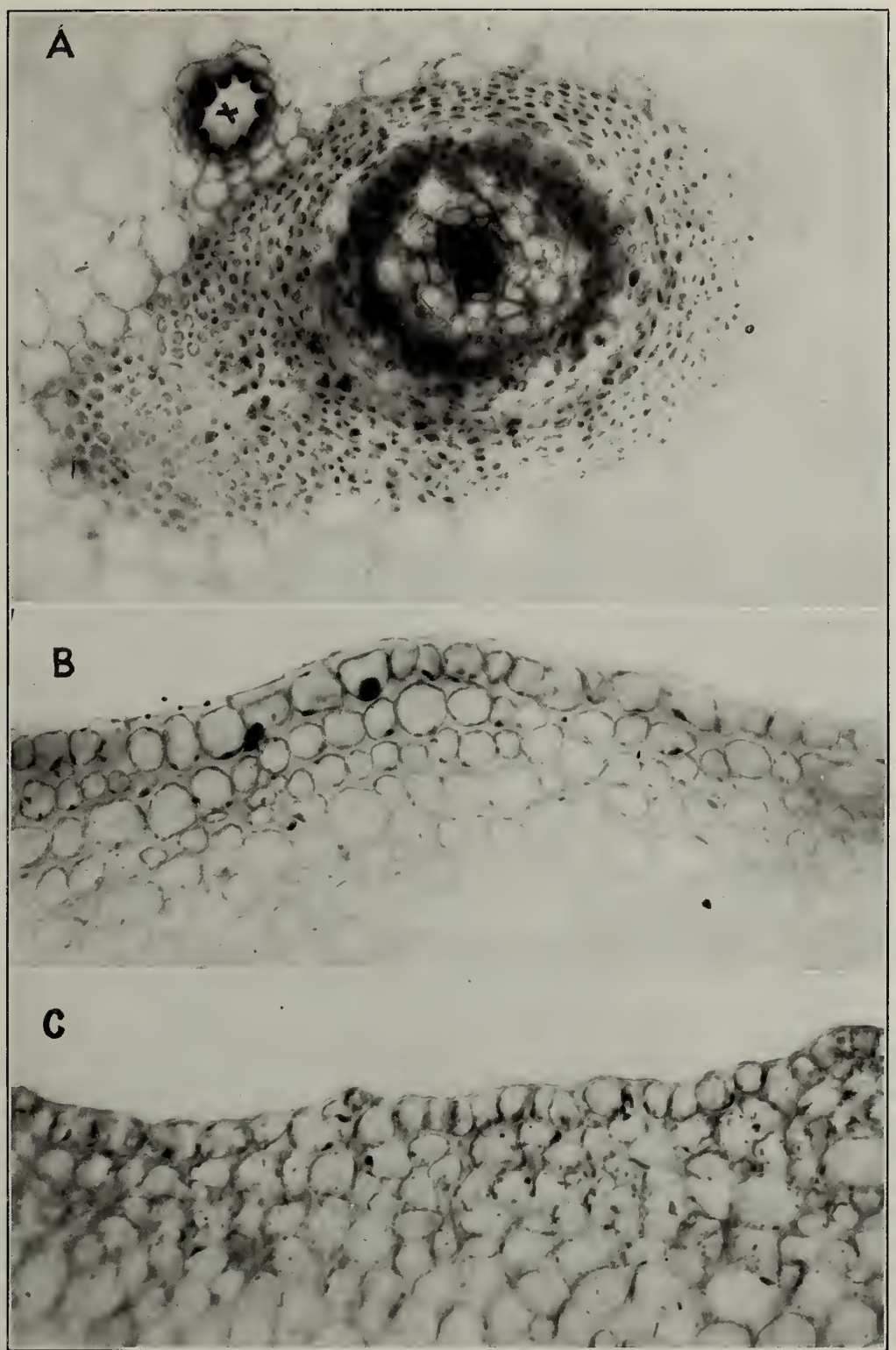
Cross section of a stem at two levels showing a well-developed pith cyst with hairy lining and subtending vascular bundles. The lower photograph was made further down than the upper and here the cyst has ruptured to the surface. Planar enlargements from fresh unstained material. Needle pricks only.

PLATE XXXIII

A. Cortex of sunflower No. 21 near one of the cysts (Pl. XI at *x*) showing in cross section a cylinder of xylem (center) entirely surrounded by phloem and that by hard bast. At *x* a resin duct showing the large secreting cells stained a deep red. These ducts are very evenly distributed near the periphery of the cortex, as may be seen on Plates VIII, XI, and XXIV. ×300, nearly.

B. A portion of the wall of one of the cysts in No. 21 (Pl. XI). In other places there are hairs. ×300, nearly.

C. Portion of the left wall of the cyst in No. 24D. See Plate XXVIII. ×500, nearly.


A
B
C

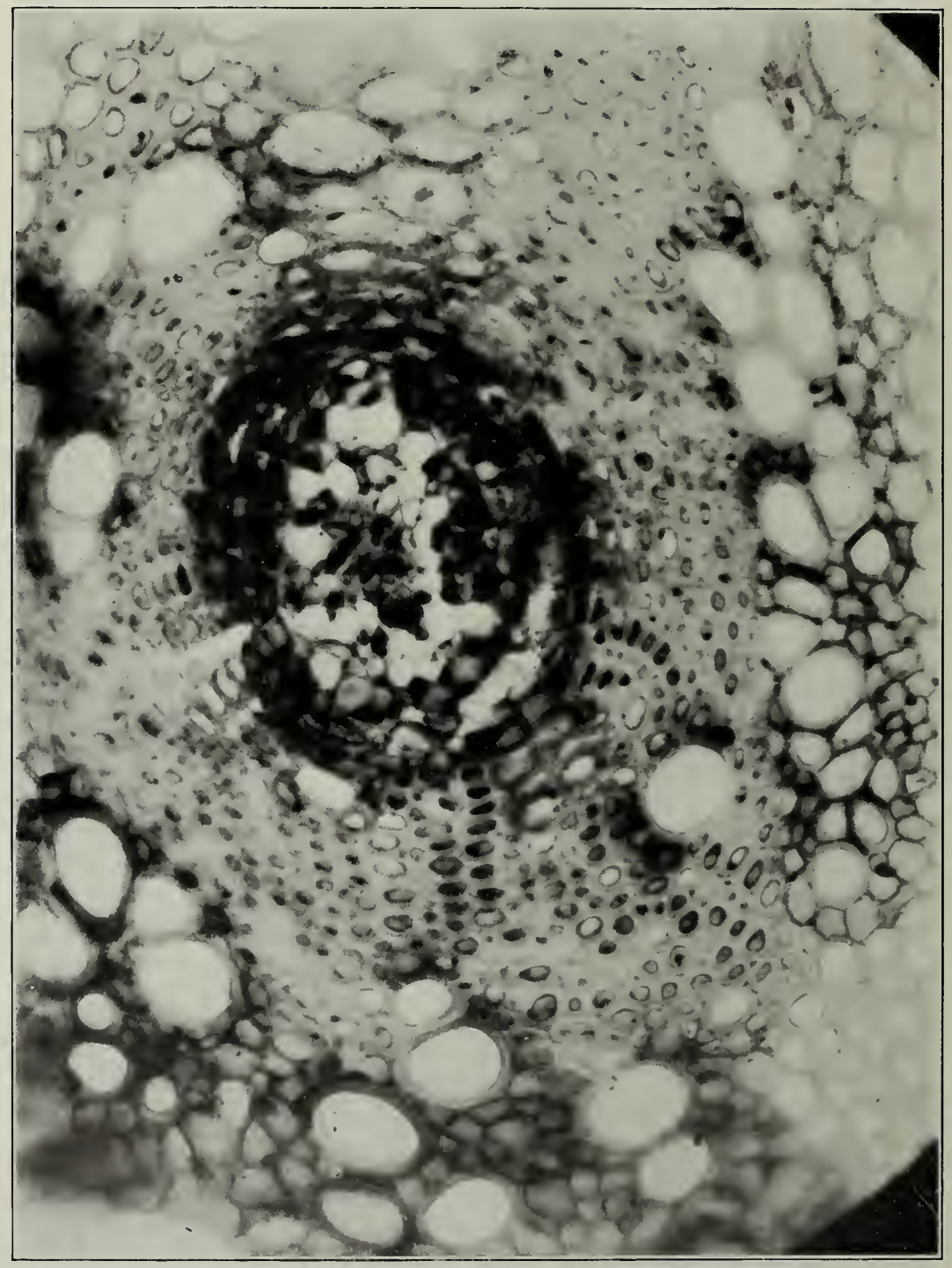
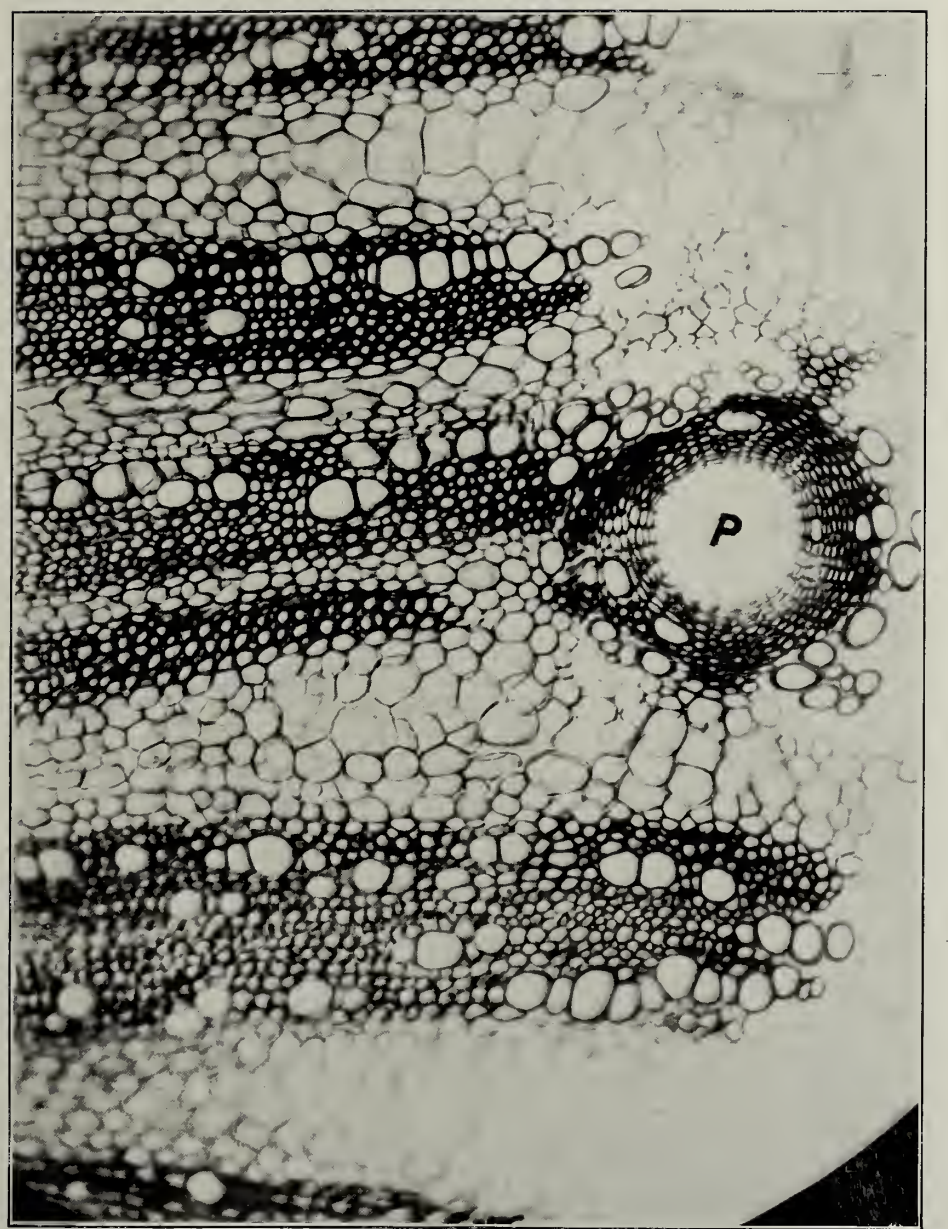


PLATE XXXIV

An abnormality in the otherwise normal vascular cylinder of No. 24F at *X*. (Pl. XXVII.) Here has developed in the middle of the inner xylem a cylinder of soft bast surrounded by cells that stain like bast fibers. Stained with methylene blue and acid fuchsin. For orientation see also Plate XXXV. ×58, nearly.

PLATE XXXV

Same as Plate XXXIV, but including a larger area for orientation. Almost the full width of the xylem is here included; phloem at *P*. Stained with pyronine and methyl violet. ×120.



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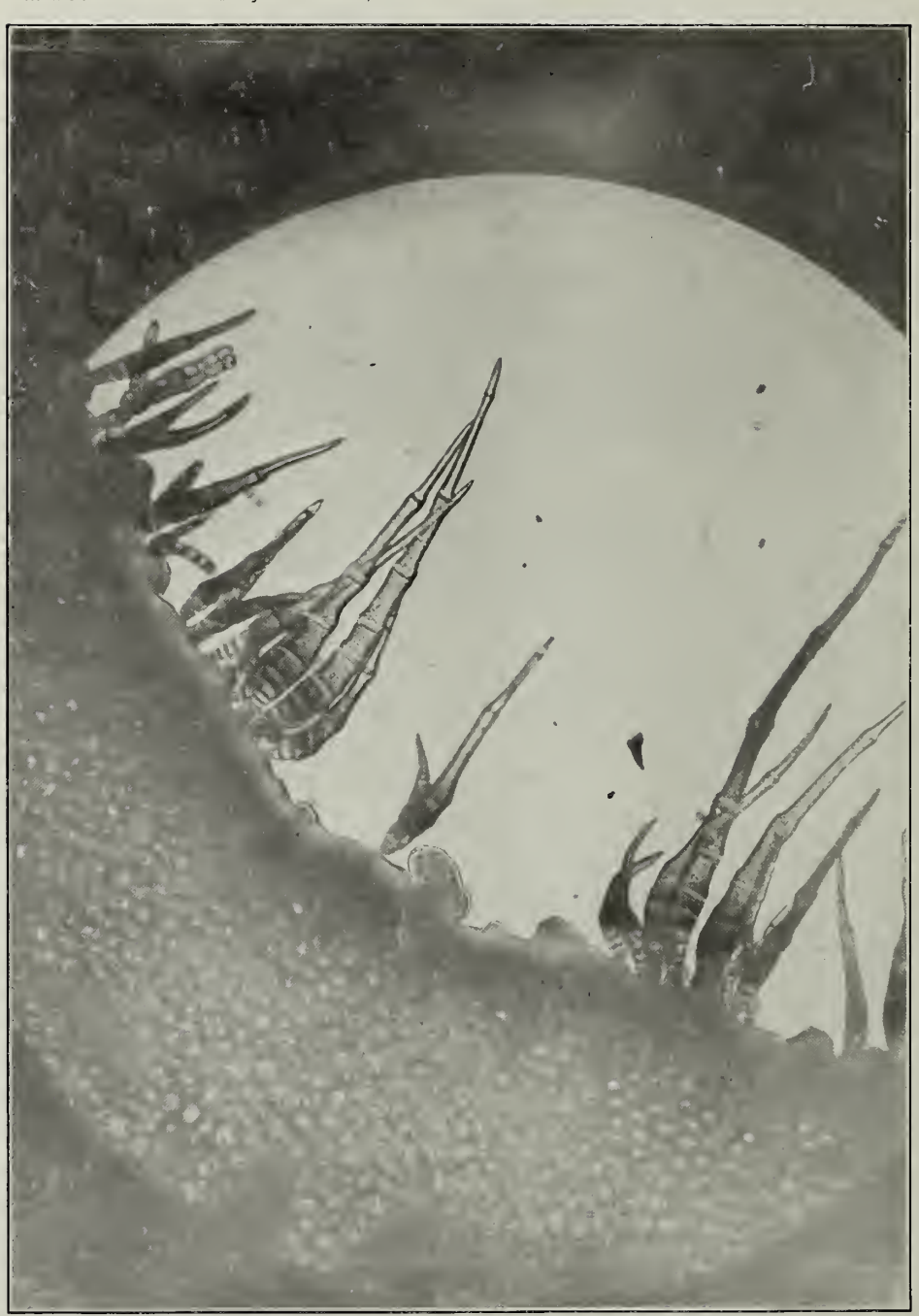


PLATE XXXVI

Types of hairs (trichomes) growing from the cyst wall of No. 24B, Plate XXVII. Cut free hand and stained with methylene blue. ×80.

PLATE XXXVII

Sunflower head from the west row wounded in the young flower head by pricks with a sterile needle, showing copious development in the center of the head of green bracts and yellow ray flowers, the latter not yet expanded. Receptacle punctured July 10, 1926. Photographed, August 12, 1926, one-half natural size. There were two cysts in the stem pith.

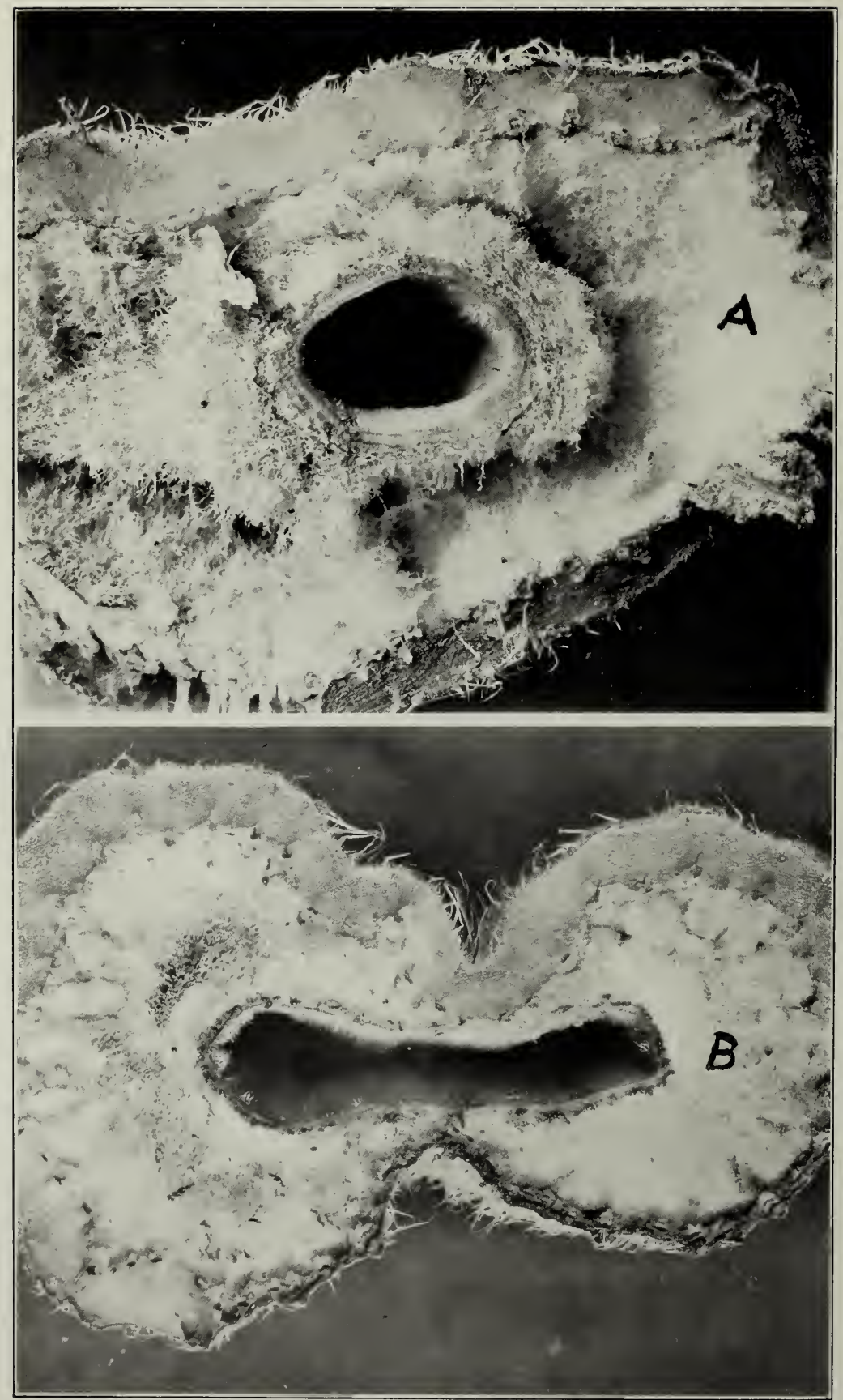

A
B

PLATE XXXVIII

A. Cross section of a sunflower stem (east row, plant No. 2) just under the flower head, showing the result of a single puncture with a blunt, sterile steel needle. In the center of the pith is a cyst subtended by a ring of vascular bundles, the order of the tissues being reversed, i. e., from within going outward: (1) Epidermis bearing trichomes, (2) subepidermal parenchyma, (3) bast fibers, (4) phloem, (5) cambium, (6) xylem, (7) normal pith of the stem. Punctured July 6, photographed September 13, 1926. ×4+.

B. East row, plant No. 11. Cross section of a sunflower stem just under the flower head showing a central cyst covered with hairs and subtended by a stele with reversed polarity. Photographed September 14, 1926. ×4+.

PLATE XXXIX

Longitudinal section of stem of plant No. 1, east row, taken 6 to 8 inches below the flower head to show continuous cyst cavity lined with trichomes, the result of one prick with a blunt, sterile steel needle into the young flower head on July 6. Photographed September 13, 1926. ×5.

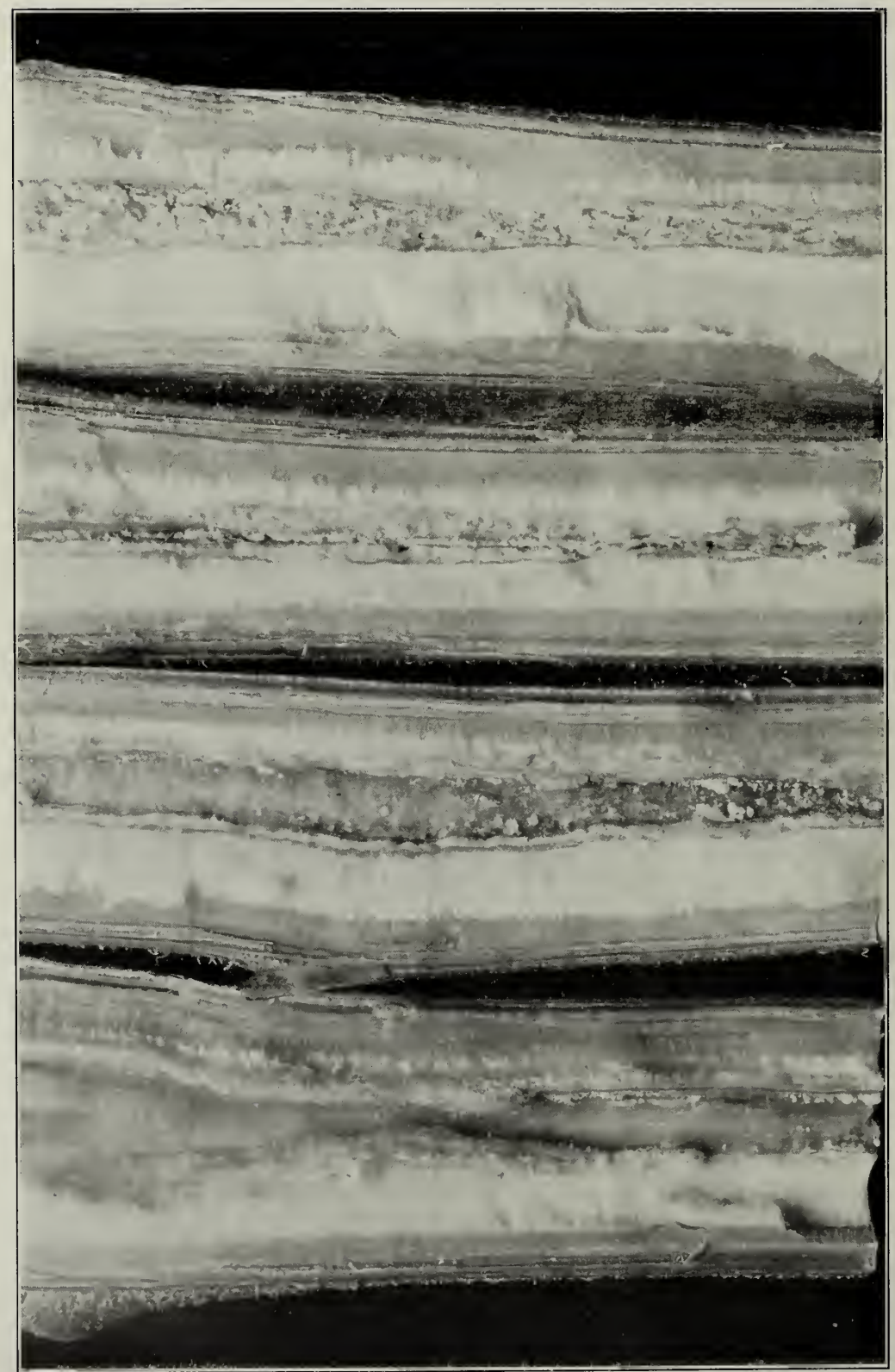


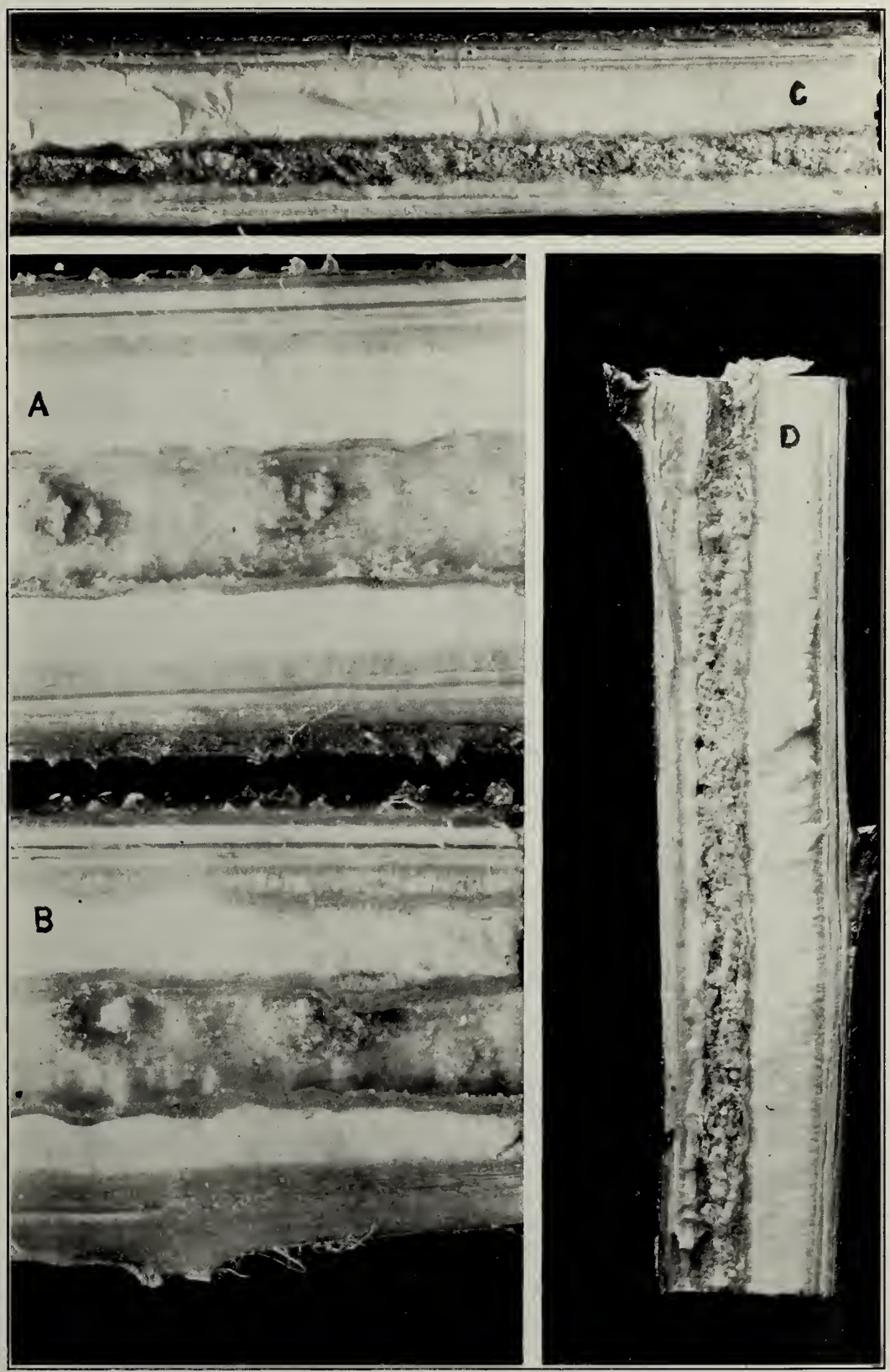
PLATE XL

Same as Plate XXXIX (east row, plant No. 1), but lower, showing another part of the continuous stem cyst. These cuts were taken 24 to 36 inches below the wounded flower head. There is pith at either side in which is embedded a continuous cylinder of reversed xylem-phloem subtending the cyst cavity. The cyst wall here bears fine granules instead of trichomes; each piece was approximately 3 inches long. Photographed September 13, 1926. ×2, nearly.

PLATE XLI

A, *B*. East row, plant No. 2. Two short pieces of sunflower stem in longitudinal section showing cyst wall bearing granulations of various sizes. These were cut about 1 foot below the head. Photographed September 13, 1926. ×4.

C, *D*. Split sunflower stem (east row, plant No. 15) taken 18 to 24 inches below the punctured flower head. *C*, upper 3 inches of the cut stem; *D*, lower 3 inches. The cyst is here full of granules and, as usual, the walls are subtended by xylem-phloem bundles with a reversed polarity. Reaction due to one blunt needle thrust made July 6, 1926. Photographed September 14, 1926. ×2.


C
A
B
D

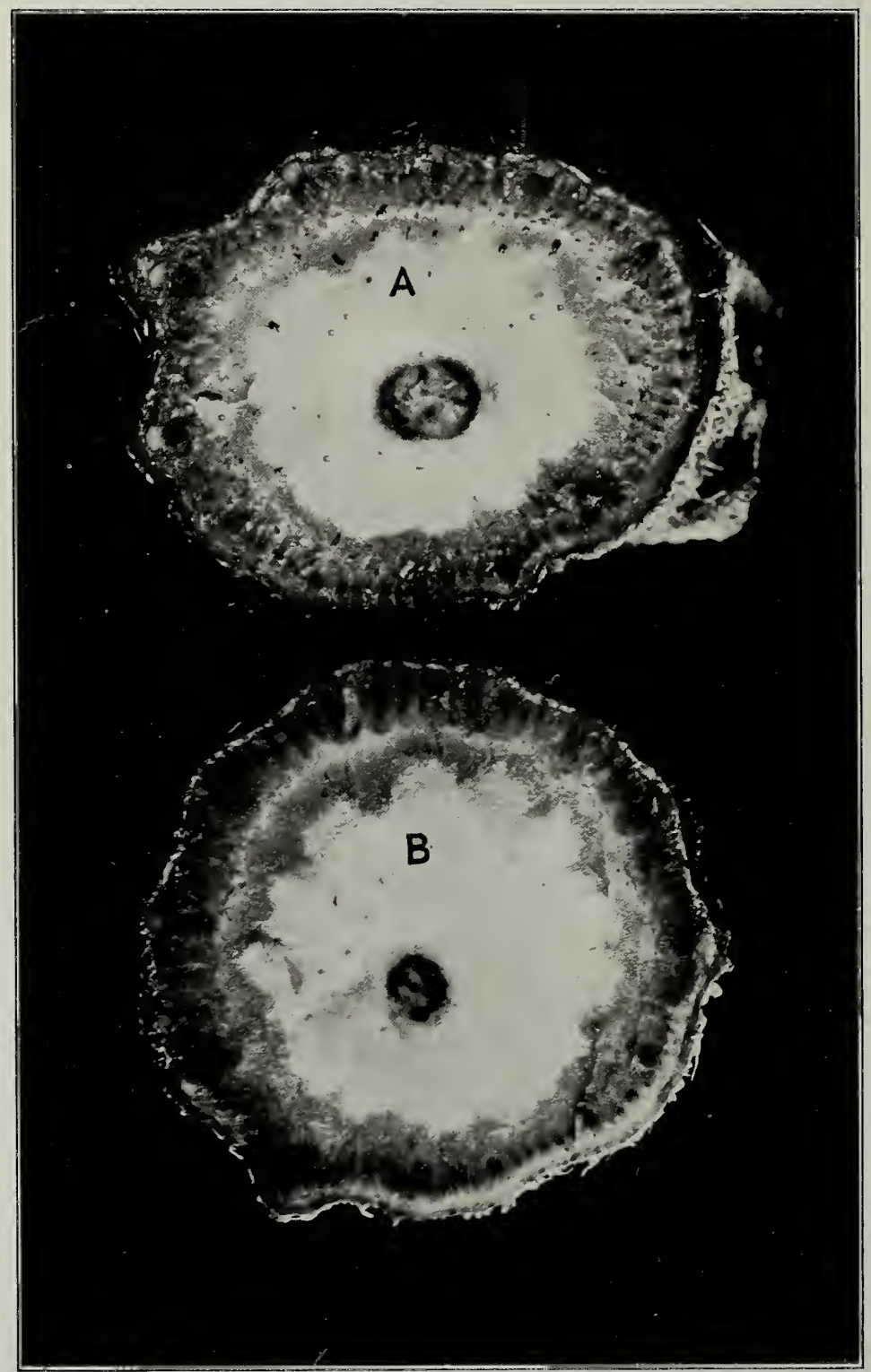

A
B

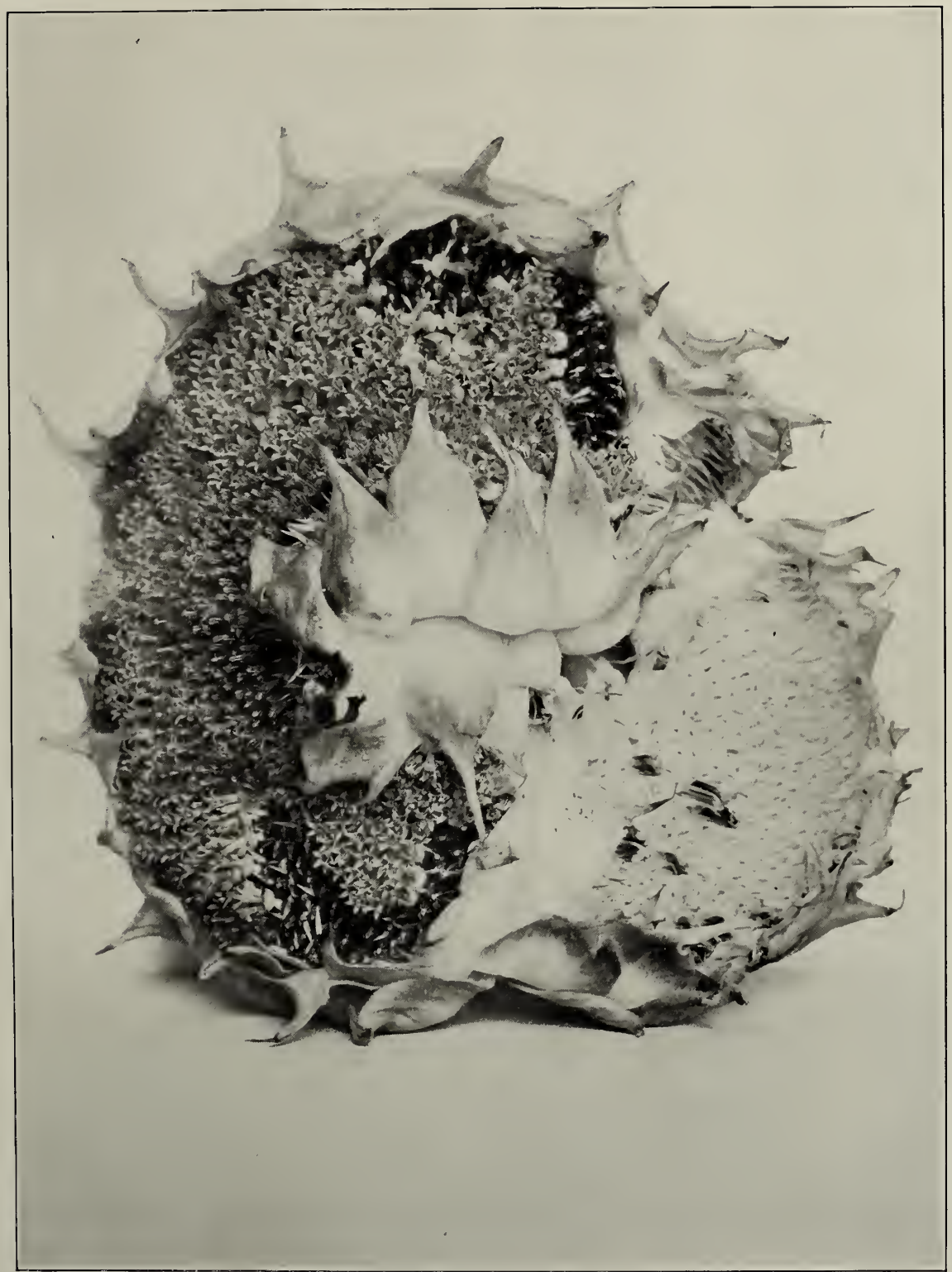
PLATE XLII

Same as Plate XLI, *A*, *B*, i. e., east row, plant No. 2, showing cross sections of stem 26 inches (*A*) and 28 inches (*B*) below the flower head. Here the cyst cavity is closed by granulations but the vascular bundles in the center of the pith remain. Photographed September 13, 1926. ×5.

PLATE XLIII

Sunflower head in fruit (plant No. 3, east row). This received a single sterile needle prick in the young flower head on July 6. It shows large green bracts in the center of the head. There were also yellow ray flowers, but these have shriveled and fallen. Photographed September 3, 1926; nearly natural size.

(All the photographs and photomicrographs illustrating this paper were made by James F. Brewer.)



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